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STUDIES

FROM THE

BIOLOGICAL LABORATORY

EDITOR:

H. NEWELL MARTIN, M. A., D. Sc., M. D., F. R. S.

ASSOCIATE EDITOR:

W. K. BROOKS, PH. D.

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ON THE ACTION OF PEPTONE IN PREVENTING BLOOD COAGULATION.

By JOHN P. CAMPBELL, A. B., Fellow of the Johns Hopkins University.

Since Schmidt-Mulheim's discovery of the fact that peptone injected into the circulation of a living animal would prevent the coagulation of its blood, if drawn shortly after the injection,[1] various hypotheses have been suggested as to what the nature of the action was. Schmidt-Mulheim himself thought that peptone did not act directly upon the substances taking part in the formation of fibrin, but that it caused something to be used up, which must be present in order that coagulation might take place. He found that the effect of peptone in preventing coagulation passed off in a comparatively short time, but that the blood still was not restored to its former condition, since a second injection of peptone had no effect until 24 hours had elapsed. Peptone disappears from the blood very quickly, and, according to his observations, none is found in the urine. He found that peptone blood was not incapable of coagulating, for if it was simply allowed to stand until putrefactive changes began, a firm clot was produced. He concludes that the peptone in some way prevented the formation of fibrin ferment.

Fano repeated and extended this work.[2] He found that 0.3 gr. of peptone to each kilo. of body weight was the most favorable quantity to inject, when all was injected at once. A much larger amount than this might be injected at intervals without affecting coagulation in the least. This was true at any rate for dogs. Rabbits, on the other hand, were not in the least affected by peptone in any quantity; and to anticipate a little, I may here say that I have found that cats likewise are not affected. Fano explained the disappearance of peptone by supposing that it entered into a combination with some element of the blood and kept it from taking part in coagulation, at the same time losing its own identity. This compound he thought was soon removed from the blood, and not enough of the original element, whatever it was,

was left to enter into a combination with a new quantity of peptone injected immediately after the effect of the first injection had passed off. He found that peptone blood might be made to clot by adding water or passing carbon dioxide through it, the latter acting much the more quickly. Peptone plasma, on the other hand, did not clot so readily under these conditions; and when the white corpuscles were very thoroughly removed it was difficult to get a clot at all. From this fact Fano concluded that the white corpuscles were directly affected, and that in some way the formation of ferment from them was prevented.

Wooldridge[4] has advanced the view that peptone prevents the interaction of plasma and leucocytes to form fibrin. He believes that when blood is drawn the plasma itself destroys the leucocytes, converting them into fibrin, and at the same time causing them to give up ferment which coagulates the fibrinogen pre-existent in the plasma. He thinks that the injection of peptone in some way takes away this power from the plasma.

Pollitzer[5] has recently shown that albumoses possess this clot-preventing power to even a greater extent than the pure peptone. He also succeeded in preventing coagulation by drawing blood into solutions of peptone or albumoses, which Fano failed to do. He makes the statement also that the cat is susceptible to the action of peptone, but that larger quantities are necessary than for dogs. He does not, however, discuss what the probable nature of the process is.

All of these hypotheses are unsatisfactory since they involve much speculation, with comparatively little experimental support. Moreover, no one of them will account for all the facts.

Further work on the subject seemed desirable in order to reconcile the discrepancies, if possible, or decide between conflicting views. Fano's idea is that peptone prevents ferment formation, while Wooldridge thinks that its action is interfered with. It occurred to me that light might be thrown upon this point by studying the effect of peptone upon the coagulation of fibrinogen under the influence of ferment. For this purpose fibrinogen was made from both cat and terrapin plasma by the ordinary method described by Hammarsten, slightly modified. Terrapin plasma was obtained by simply letting the blood stand in a refrigerator. Cat plasma was in one or two cases gotten in

the same way, but this was uncertain, so I generally used $MgSO_4$ plasma. An equal volume of saturated NaCl solution was added to the plasma, and in a few minutes an abundant precipitate was formed. This was filtered off, pressed, and dissolved in about one-third of the original volume of weaker NaCl solution (8 per cent.). To this was again added an equal volume of saturated NaCl, and the precipitate was again dissolved as before, but this time in distilled water, the salt clinging to the paper being sufficient to effect the solution. Fibrinogen prepared in this way is perfectly free from paraglobulin, since after heating it to 57° C. for a few moments all the proteid present is precipitated.

There seemed to be considerable difference in the proportion of fibrinogen present in these two sources, cat plasma being much the richer. The original precipitate gotten by saturating cat plasma with salt was certainly much more abundant than that gotten from terrapin plasma, and starting with equal volumes of plasma from the two sources, and subjecting them to the same treatment, the resulting solution of fibrinogen from the cat plasma will give a much greater heat coagulum than the plasma from the terrapin. This point I did not decide quantitatively, but simply from the amount of space occupied by the precipitates after settling. Fibrin ferment was made by Schmidt's method essentially, but as I found a slight modification by which a ferment which is very active and at the same time very free from albumin could be gotten in a short time, it may be worth mentioning. Alcohol is added to perfectly fresh ox serum in equal volumes. After standing 24–48 hours, the coagulated proteid is freed from alcohol by filtration and then extracted with water at a low temperature for about twelve hours, the volume of water being equal to that of the original serum. The water, containing the ferment and considerable albumin in solution, is filtered off into a large volume of alcohol. After standing two or three days, this may be filtered off and the coagulated proteid simply spread out evenly over the filter paper and allowed to dry. Extracting a small piece of this paper with water gives a ferment which is very active, and gives on boiling hardly a perceptible opalescence. A second extraction of the original proteid will also give an active ferment.

Some of the peptone used was made according to the method described by Maly,[6] and some was commercial peptone made by Merck. The latter was strongly acid, and contained albumoses in considerable quantity, but there was also much peptone present.

In order to see whether all or part of the clot-preventing power of peptone was due to action upon the fibrinogen, and also whether any differences existed in fibrinogen from different animals, experiments were made, from which the following are selected as examples.

Experiment No. 3.

Fibrinogen was made from terrapin plasma, and the peptone was some which I made myself. It was neutral, and dialysed free from salts, but contained albumoses. Six test-tubes were prepared as follows:

No. 1. 2 cc. fibrinogen solution + 2 cc. water.
No. 2. " " " + 2 cc. ferment solution.
No. 3. " " " + " " " + 2 cc. 10% peptone solution.
No. 4. " " " + " " " + 1 cc. " " "
No. 5. " " " + ½ cc. dog serum.
No. 6. " " " + " " + 1 cc. 10% peptone solution.

These were kept at 38° C. In a few minutes No. 2 clotted, and within half an hour No. 4. No. 5 clotted some time during the night, and Nos. 1, 3 and 6 not at all.

Experiment No. 9.

Fibrinogen was made from cat plasma which was gotten by simply cooling the blood. The various solutions were added in exactly the same proportion as in the experiment just quoted, and the six test-tubes were treated in the same way. Here, however, a different result was gotten, for it was found that twice the volume of 10 per cent. peptone solution was not sufficient to prevent coagulation, other conditions being exactly the same as in the former experiment.

This appeared to indicate the existence of some fundamental difference between the specimens of fibrinogen gotten from the two sources, and, taken together with the fact that peptone injected into a cat is without effect, seemed significant. A

number of other conditions, however, must be taken into account before we can draw any such conclusion. It is perfectly natural to suppose that quantitative differences may exist in the elements of the blood of different animals; but we would hardly expect to find qualitative differences of a fundamental nature in a constituent like fibrinogen, which conducts itself in so many respects in the same way whatever be the source from which it is obtained.

We must also take into account the strength of the ferment used. In Experiment 3 we find that an equal volume of 10 per cent. peptone solution is sufficient to prevent the action of strong ferment, while half the volume is not. On the other hand, half the volume is sufficient to prevent the action of dog serum, which, however, acts much more slowly than the pure ferment solution. The fact remains, however, that if we take two specimens of fibrinogen made in exactly the same way, and add to each the same quantities of peptone and ferment, that made from the cat will clot, while that from the terrapin will not.

It has been stated above that fibrinogen is more abundant in cat's blood than in terrapin's. Accordingly, I made a new supply of fibrinogen from cat plasma, using this time $MgSO_4$ plasma, and getting, finally, a solution which seemed about as strong as that ordinarily gotten from terrapin plasma. With this I made the following experiment.

Experiment No. 13.

In this Merck's peptone was used, dissolved in 5 per cent. $NaHCO_3$ until a neutral solution was gotten, and then diluted up to 10 per cent. with distilled water. Ten test-tubes were arranged as follows:

	Fibrinogen solution.	Ferment solution.	Peptone solution.	Water.
No. 1	1 cc.	1 cc.	.....	1 cc.
No. 2	"	"	.....	2 cc.
No. 3	"	"	1 cc.	"
No. 4	"	"	"	1 cc.
No. 5	"	"	.....	.....
No. 6	"	.....	.....	1 cc.
No. 7	"	"	1 cc.	.....
No. 8	"	"	1 cc. 5% sol.	.....
No. 9	"	"	½ cc. 5% sol.	.....
No. 10	2 cc.	"	1 cc. 10% sol.	.....

In one hour these were found as follows: No. 1 clotted firmly; No. 2 clotted, but the clot very loose and soft; No. 3 no clot; No. 4 no clot; No. 5 clotted firmly; No. 6 no clot; No. 7 no clot; No. 8 beginning to clot; No. 9 clotted tolerably firm; No. 10 no clot.

During the night Nos. 4 and 7 clotted, and after about four days No. 10, but No. 3 stood for a week without any signs of coagulating.

Here, then, we get almost the same results as with terrapin fibrinogen. It appears, then, that there is probably no fundamental difference in fibrinogen gotten from these two sources, but that it is only a difference in its quantity which caused the difference in result in the previous experiments. The action is a many-sided one, and the strength of each of the three elements must be taken into account. In the last experiment, No. 3, which never clotted, differed from No. 4, which clotted only after a long time, in containing 1 cc. of water. This of course weakened the ferment and diluted both the peptone and fibrinogen. Comparing Nos. 1 and 4 with Nos. 2 and 3, we see that the effect of peptone is most marked in No. 3, where the dilution is greater, and where the coagulating time, if peptone were absent, would be longer.

In attempting to account for the effects gotten by injecting peptone in this way we meet with difficulties. The normal time for the blood of any animal to coagulate when drawn is shorter than that of fibrinogen with even the strongest ferment. Consequently, for a given volume of blood we would expect even more than twice its volume of 10 per cent. peptone solution to be necessary in order to prevent coagulation, if its only effect is to prevent the interaction between fibrinogen and ferment. This quantity is much greater than is necessary when peptone solution is injected into the blood-vessels.

If blood be drawn into a solution containing enough peptone to afford a proportion which would act on the circulating blood, it will coagulate almost as usual. On the other hand, drawing blood from either the dog or terrapin into an equal volume of 10 per cent. peptone solution will generally prevent coagulation, though this is not invariable.

Peptone must then do more than merely prevent the action of ferment when injected into an animal. Moreover, certain

phenomena observed after peptone injection remain to be accounted for. The disappearance of peptone is not explained; and even supposing this to be not at all connected with any element of the blood, we still cannot account for the failure of a second injection made as soon as the effect of the first has passed off.

If, then, peptone does not merely prevent ferment action, can it in any way prevent ferment formation? Wooldridge claims that no ferment exists in peptone blood, since, by coagulating the proteids with alcohol and extracting with water, he did not get an active ferment. Without putting too much confidence in a negative experiment like this, other evidence may be brought forward in support of the correctness of the view. As already stated, peptone blood may be made to clot in various ways, as by the addition of water or CO_2, or by putrefactive processes. Peptone plasma may be made to clot in the same ways, but not so readily, and the more thoroughly the corpuscles are removed the more difficult the clotting. Peptone plasma from the terrapin, in which the corpuscles settle very thoroughly, may be allowed to stand indefinitely and putrefaction will not cause it to coagulate. Schmidt-Mulheim believes that in putrefaction a ferment is furnished which directly causes the coagulation of the blood and takes the place of fibrin ferment. Against this may the urged the fact that putrefaction will not cause the coagulation of fibrinogen solution, nor will it cause plasma to coagulate from which all the corpuscles have been very thoroughly removed. The same is true of the addition of water or CO_2. We must assume, then, that the action of these substances is upon some element of the blood which can yield ferment, but which has been prevented from so doing by the peptone. But we have the disappearance, or, at any rate, the partial disappearance, of peptone from the circulating blood to account for, also the failure of a second injection to prevent coagulation when made soon after the effect of the first has passed off.

Until it is definitely settled where fibrin ferment is formed, we cannot do more than speak provisionally in regard to ways in which its formation might be prevented. The theory that ferment comes from plaques is certainly coming more into favor, but Schmidt's theory cannot yet be said to be entirely supplanted, since the coagulation of lymph, in which plaques probably do not exist, cannot be explained unless we suppose

the leucocytes to take some part in it, neither can we explain why it is that lymph from a peptonized animal will not clot unless an element common to both blood and lymph is involved.

Bizzozero[7] states that in peptone blood the plaques are perfectly well preserved until coagulation begins, when they break down, just as they do in normal blood. In blood that is not quite thoroughly peptonized I have seen large granular masses having the plaques broken down on one side and quite distinct on the other. Where they were broken down fibrin was found radiating out in abundance, but where they were distinct there was little or no fibrin. It cannot be regarded as absolutely proved that changes in the plaques are not produced by ferment, instead of being simply associated with its formation.

On Schmidt's theory it seems to me perfectly possible to explain the action of peptone, and many analogies and considerable positive evidence may be brought forward in support of this view. Hofmeister[8] claims that whenever free peptone gets into the blood some of it almost immediately appears in the urine. The proportion depends upon circumstances; for, if he injected a small quantity (not sufficient to prevent coagulation), he got back as much as 80 per cent. in the urine. The rest he thought was taken up in certain of the tissues. If, however, he injected sufficient to prevent blood coagulation, he got back only about 30 per cent. in the urine. While it must be admitted that there are no satisfactory methods for the accurate determination of peptone, differences so great as these are probably significant; they indicate that in the process of preventing coagulation some of the peptone is used up. The most natural explanation of this disappearance seems to me to be that the leucocytes directly take up the peptone, and in consequence are themselves so changed as to prevent whatever processes ordinarily take place resulting in the formation of ferment.

Schäfer has shown clearly the important part played by leucocytes in the absorption of fat,[9] and he thinks it very probable that other digestive products are taken up in the same way. Hofmeister strongly advocates the view that leucocytes in the subepithelial tissue of the digestive tract directly take up peptone. Whether all these absorptive leucocytes take the same course is uncertain; those which contain fat-drops undoubtedly get into the chyle and there disintegrate, but the fate of those

which carry other products which cannot be followed by the microscope is not so easily determined. At any rate, the number of leucocytes in the blood is greater during digestion than at other times, and this seems to indicate that they are in some way connected with the process of absorption. From the fact that cells containing fat-drops disintegrate in the chyle we can argue nothing as to the fate of cells containing peptone, since leucocytes may not be able to digest fat, while they probably can assimilate peptone.

We are hardly in a position to assume a different faculty in leucocytes, by which those containing peptone travel into the blood capillaries while those containing fat get into the chyle; but if leucocytes do play this part in absorption, and if it be true, as generally supposed, that peptone goes by the portal system, we are compelled to make such an assumption. If this be so, we cannot argue from the fate of leucocytes which carry fat to the chyle as to the fate of those which carry peptone to the blood, since the two media in all probability present different conditions.

The absence of dissolved peptone from the blood during digestion is certainly most readily accounted for in this way, and if this view is correct, we would also look for differences in leucocytes. We would expect those which have just come from the digestive tract laden with digestive products to be different from those which have been for some time in the circulation and have more or less assimilated or altered their absorbed matters. Those who hold to the view that ferment comes from leucocytes maintain that only a certain form is concerned, and that other varieties have no share in the process. Now, whatever be the origin of different forms of leucocytes, there is probably no fundamental difference between them; and any apparent differences may be explained on the supposition that their states of nutrition are different. *If it is possible for the leucocytes that have worked over their supply of food material to be gotten back into the condition of those which do not undergo change to form ferment, the action is explained.* This does not seem at all impossible. Metschnikoff's theory of intracellular digestion is strongly in support of the view that leucocytes may pick up peptone, at any rate if any considerable amount were free in the blood, and Greenwood's observations also favor this view.[10]

Here we see why a considerable amount of peptone must be injected at once, for in very slow injections the kidneys will excrete it as fast as it goes in, and the leucocytes will not be called into play at all. There is also some pathological support for this view. Hofmeister has shown that peptone is contained in pus, and that it is held by the corpuscles and not dissolved in the serum. He found that anything which caused the cells to disintegrate set free the peptone. Now, during pus formation, peptone is found in the urine, and this Hofmeister takes to indicate that, owing to the disintegration of the cells, free peptone has got into the blood.

Another point which seems to favor the view that leucocytes are acted upon is the effect of peptone upon the proteids of the plasma. By parallel determinations I have found that the total proteids of peptone plasma are greater than normal. The determinations were made as follows: A terrapin was bled into a tube packed with ice, and a measured portion of the blood thoroughly mixed with an equal volume of perfectly neutral 10 per cent. peptone solution, and allowed to stand at the temperature of the room usually about 15 hours. By this time the corpuscles had settled, leaving the plasma above perfectly clear. The determinations were made by dropping measured quantities of plasma (10 cc. of the peptone plasma and 5 cc. of the normal) into about 50 cc. of boiling water to which 5 cc. of a saturated solution of ammonium sulphate had been added. This was made faintly acid with acetic acid and boiled gently for a few minutes. The precipitate was collected on a weighed filter, washed thoroughly with water and hot alcohol, and then dried and weighed.

My figures calculated for 100 cc. of blood are:

Total proteids in normal plasma..... 2.834 gr.
Total proteids in peptone plasma..... 3.082 gr.[1]

Now, Schmidt-Mulheim found no difference in the total proteids of dog's blood peptonized inside the body; and Fano[3] found a diminution which he took to indicate that some of the proteids were used up in the process. The results gotten by the former are probably due to the fact that the very minute increase which

[1] These results are lower than those gotten by Howell (Stud. Biol. Lab. Vol. II, p. 52) for normal terrapin blood, but there is considerable variation in individual cases.

could be expected under the conditions in which he worked would be quite within the limits of experimental error. Fano's results I cannot explain, since he does not give his method of making the determinations, but from some of my earlier experiments I think it possible that his peptone solution may have been acid, and thus caused the formation of acid albumin. Fano also analyzed normal serum, and from this calculated the amount of proteid in normal plasma. This result he compared with peptone plasma, but of course little confidence can be put in this method. With the conditions under which my work was done there was opportunity for the leucocytes, if they possessed at all the power of picking up peptone and converting it over into coagulable albumin, to exercise this power to the utmost. I have not tried the effect of allowing the blood to stand in contact with peptone for varying times, consequently I am not able to say what the rate of transformation is, nor have I tried the effect of adding peptone to plasma free from corpuscles. It seems to me, however, that the inefficacy of a second injection following the first at a short interval must be in some way connected with the fact that the leucocytes are not in a condition to take up and work over a second supply until after a certain time has elapsed. On the other hand, the increase in total proteids may also have its effect, for in cat's blood, in which the percentage of total proteids is high, even the first injection does not prevent coagulation.[1]

The tendency to coagulate shown by cat's blood is very strong, other anti-coagulants which work perfectly well on dog's blood being without effect on cat's. Heycraft's "leech extract"[(11)] does not at all prevent coagulation in cat's blood. That this is to be explained by any differences in the leucocytes seems to me unlikely; more probably, in some way the large percentage of coagulable albumin in cat's blood is connected with the different result.

[1] In this point I am unable to confirm Pollitzer's statement. I have made about ten attempts, using peptone, which worked perfectly well on dogs, and varying the quantities. 4 gr. injected into a cat weighing 1.22 kilos was without effect on the blood, although the physiological effects, anaesthesia, etc., were very marked. I have in single cases succeeded in delaying the coagulation of cat's blood by injecting peptonized dog's blood. In one case an injection of 160 cc. of dog's blood into a cat weighing 3.3 kilos produced unconsciousness, and kept the blood from coagulating for nearly an hour.

To recapitulate:

1. The mere presence of peptone is unfavorable to the formation of fibrin from fibrinogen, and this influence is the more marked in proportion as the time of coagulation is greater.

2. The above fact will not account for all the phenomena. The proportion of peptone which will work inside the body will not prevent coagulation when added to the blood outside the body, and the disappearance of the peptone is not explained, nor is the inefficacy of a second injection.

3. A larger proportion is needed outside the body because there its effect is to prevent the action of ready-formed ferment, while inside the body it may prevent ferment formation.

4. What occurs is probably as follows: If peptone be injected slowly, much of it is excreted by the kidneys. If a large quantity is injected quickly, the leucocytes, or some of them, will directly take up the greater part of it, and in this act they are so changed as to be incapable of taking part in blood coagulation.

5. As soon as the circulating blood has regained its coagulating power a fresh peptone injection is inefficient, because the leucocytes have not yet used up the last supply, and have, so to speak, as yet no appetite for more.

In conclusion, I wish to express my thanks to Prof. Martin and Dr. Howell for many valuable suggestions.

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NOTE ON THE SPECIFIC ENERGY OF THE NERVES OF TASTE.

By W. H. HOWELL, Ph. D., and J. H. KASTLE, S. B.

It is generally admitted amongst physiologists at present that our taste sensations can be reduced to the four primary sensations of sweet, bitter, acid, and salty. Of these, sweet and bitter are pure gustatory sensations, that is, they result from stimulation of the taste nerves alone, while acid sensations, unless the acid used is very dilute, are accompanied by sensations caused by the simultaneous stimulation of the nerves of general feeling in the tongue; and the same is true, though to a less extent, of salty taste sensations. All other taste sensations that we are accustomed to distinguish may be regarded as resulting from the combination of two or more of these primary sensations, or from a union of one or more of the taste sensations with the ordinary common sensations or touch sensations of the tongue.

Granting that we have these four primary taste sensations, it becomes a question whether or not each is mediated by its own set of nerve fibres. That is, may one and the same nerve fibre give at one time a sweet and at another time a bitter or acid sensation according to the character of the sapid body used, or does each nerve fibre when stimulated, if it responds at all, give only one sensation? The latter supposition is the one usually accepted, inasmuch as all the phenomena known with regard to taste sensations are readily explained by its aid; so that it is customary to speak of sweet nerve fibres, bitter nerve fibres, etc., in the same sense as we speak of red nerve fibres and green nerve fibres amongst the visual nerves, or of heat nerves and cold nerves in describing the temperature sensations. While the doctrine of specific nerve energies seems to meet all the requirements, at least better than any other theory, no good proof of its correctness with regard to gustatory sensations has yet been offered. It is known that the taste sensations of different parts of the tongue differ from one another, and, moreover,

that in certain persons one portion of the tongue may be insensitive to one or more of the different tastes — facts which point to the doctrine of separate nerves for each taste. But perhaps the best probable proof would be obtained if it could be shown that one substance, chemically pure, is able to arouse distinctly different sensations of taste upon different parts of the tongue. Very many observations of this kind have been made by Horn, Picht, Guyot, Lussana and Inzani upon a great many different substances, but the results as tabulated by v. Vintschgau in the third volume of Hermann's Handbuch der Physiologie show that they have met with but little success. The results obtained by the different observers do not agree amongst themselves, and in no case was a substance found which gave distinctly different taste sensations upon different parts of the tongue. Perhaps the most satisfactory substance obtained was sodium sulphate, which, according to Guyot, tastes distinctly salty upon the tip of the tongue and distinctly bitter upon the back, and Lussana and Inzani also report the same difference. But Horn, and also Picht, state that this substance tastes acid upon the tip of the tongue and bitterish and salty upon the back.

During the past session a substance was brought to our attention which shows this peculiarity in a very distinct way, and the observations that we made upon it seem worth recording, since it is, perhaps, the best single proof of the specific energy of the taste fibres. The substance in question goes by the name of para-brom-benzoic sulphinide, and is one of a group of bodies made in the chemical laboratory of this University under the direction of Prof. Remsen, and, as far as we know, not made elsewhere. The chemical structure of the body may be represented by the formula

$$C_6H_3Br \begin{cases} CO \\ SO_2 \end{cases} > NH,$$

being the bromine derivative of benzoic sulphinide, which has the formula

$$C_6H_4 \begin{cases} CO \\ SO_2 \end{cases} > NH.$$

This latter substance is intensely sweet, and has lately come into the market as a substitute for sugar under the name of saccharine, while the bromine compound upon which most of our observations were made is sweet upon one part of the tongue and

bitter upon another part. The sample used by us was chemically pure, and the general result of our experiments showed that on the back of the tongue, *i. e.* in the region of the circumvallate papillae, along the borders of the posterior half of the tongue, and on the soft palate, it gives a very strong and pure bitter taste, while on the tip and along the borders of the anterior half of the tongue it gives a sweet taste.

The substance was tried upon about twenty persons, although the most careful experiments were made upon ourselves. In experimenting, we usually had three solutions — one of sugar, 10 per cent., one of quinine, and one of the brom-sulphinide—which were warmed to the temperature of the tongue to prevent the sensation of a cold body which would otherwise be aroused. The person experimented upon had one of these three solutions, which one of course he did not know, dropped upon a given portion of his tongue by another person. The portion of the tongue stimulated was not allowed to touch any other part of the mouth cavity. The sensations aroused were written down and numbered, and afterwards compared with the list of substances used by the person experimenting. Our results were always the same as far as the tip and the circumvallate region of the tongue were concerned. Upon the latter the brom-sulphinide caused an intense bitter taste, while upon the tip it caused a distinct, though not very strong, sweet taste, which was sometimes reported simply as sweet, at other times as metallic sweet, or sweet and slightly acid. It may be well to state that both of us have a strong sensation of bitter upon the tip of the tongue when stimulated by quinine. Upon the borders of the anterior half of the tongue the sensation caused by the brom-sulphinide was the same as upon the tip, while along the borders of the posterior half the sensation was an intense bitter. Upon the middle of the dorsal surface of the tongue no taste sensation at all was aroused, nor upon the hard palate. Upon the soft palate the results were somewhat variable; the sensation was sometimes described as bitter alone, sometimes as sweet, or sweetish quickly followed by a strong bitter.

The responses made by the other persons upon whom the substance was tried were somewhat variable. All of them stated that upon the posterior part of the tongue, circumvallate region,

the sensation was very intensely bitter, equal to or stronger than that caused by quinine. Upon the tip of the tongue some of them described the sensation as "metallic or acid sweet," "sweet and slightly astringent," while most of them described it simply as sweet or sweetish, though when questioned they usually added that it was slightly astringent or metallic; so that in most cases without doubt the brom-sulphinide on the tip of the tongue stimulates the nerves of general sensation as well as the "sweet nerves." The sweet sensation on the tip was always much more distinct if the tongue was rubbed gently against the back of the teeth, as we would expect.

In several of the persons experimented on the sensation upon the tip of the tongue was given as first slightly bitter and then sweet, and we ourselves toward the end of the experiment were generally able to detect both these sensations in the order named, especially if we knew that the brom-sulphinide was to be used. This seems rather curious when we remember that the reaction time of the tip of the tongue for quinine, according to the experiments of v. Vintschgau and Hönigschmied, is decidedly greater than for sugar. One would have supposed that in this case, if the substance was capable of causing both a bitter and a sweet sensation on the tip of the tongue, the sweet sensation would have been developed first. The fact that it was not seems to show that the reaction time as determined for quinine and sugar respectively cannot be accepted as true for all other bitter or sweet substances. We have met with but one person upon whom this substance has failed to give both a sweet and a bitter taste in the places described. In this case the sensation caused upon the circumvallate region was, as in other persons, intensely bitter, while upon the tip of the tongue the sensation was described sometimes as slightly bitter, sometimes as salty and bitterish. This was not owing to a lack of sensitiveness to sweet substances on the tip, since sugar gave a decidedly sweet sensation.

It seems to us that these results show beyond doubt that a chemically pure substance can arouse different taste sensations upon different parts of the tongue, a fact which goes a long way towards proving that each taste sensation is provided with its own set of nerve fibres. The action in this case is more interesting,

perhaps, in that the two sensations aroused, sweet and bitter, are those which are universally acknowledged to be pure taste sensations. The results obtained by us can be explained by supposing that the brom-sulphinide stimulates both the bitter and the sweet fibres, and that upon the posterior portion of the tongue, where, as is well known, the bitter taste is most developed, the stimulation of the bitter fibres is so strong that only this sensation is perceived, while upon the tip of the tongue, where the bitter fibres are less numerous, the sensation aroused by the stimulation of the sweet fibres predominates, though, as was stated, several persons were able to recognize both sensations.

We had intended to try the same substance upon some of the lower animals and determine what effect would be produced by section of one or other of the gustatory nerves. We were unable, however, to get an animal whose sense of taste was sufficiently delicate to lead us to suppose that any certain results could be obtained. Although the brom-sulphinide has such a disagreeably bitter taste upon the posterior part of the tongue in man, neither it nor quinine seemed to have much effect upon the animals we used—dogs, rabbits and cats—if one could judge their sensations from their actions. Our experience with these animals has led us to doubt very much the value of the experiments made by various observers upon dogs with regard to the effect of section of the glossopharyngeal or lingual nerves upon the sense of taste in different parts of the tongue or palate.

We made a number of experiments also upon the benzoic sulphinide, saccharine, the substance from which the brom-sulphinide is derived, which gave us somewhat interesting results. With some persons this substance arouses only a very sweet sensation, whether placed on the tip or the root of the tongue. With others, however, while it caused a strong sweet sensation upon the tip, when placed upon the root in the region of the circumvallate papillae it caused first a sweet, then a bitter sensation, and frequently the two sensations rapidly alternated with each other, reminding one very much of the conflict of the visual fields when two different colors are thrown upon corresponding points of the two retinas; the stimulation of the two kinds of taste fibres in this case being so strong that neither one could overshadow the other, and the mind perceived first one, then the other.

THE INFLUENCE OF WARMTH UPON THE IRRITABILITY OF FROG'S MUSCLE AND NERVE.

By CHARLES L. EDWARDS, A. M. With Plate IV.

Historical.

At the suggestion of Dr. Howell the following experiments were undertaken to demonstrate the effects of warmth upon the irritability of frog's muscle. The historical resumé is brief, for although a number of investigators have given hints, only two, Marey and Schmulewitsch, have given us important results concerning this phase of muscle activity.

Helmholtz,[1] whose classical researches have furnished the foundation for the modern investigation of muscle phenomena, seems not to have mentioned the influence of warmth, although he noticed that of cold, upon muscle contraction. E. J. Marey,[2] in 1868, with new and much improved methods, was enabled to record very elaborately his results.

The frog's gastrocnemius was placed in a metallic case surrounded by a second case, while in the annular space between the two warm water was made to flow. The effects of heating the muscle he divided into two periods. In the first, the period of "excitation of muscular action," the simple contraction is shorter and stronger as the temperature rises, provided, however, it does not exceed 30° to 35° C. In the second period, that of "alteration of the muscle by warmth, and loss of irritability," if the warmth be increased from the previous limit of 35° C., the height of the contraction decreases and the muscle does not regain its normal length, so that each contraction curve shows a marked contracture. If the muscle is warmed by submitting it immediately to a temperature of 45° to 50° C. this result of the second period may be obtained at once without the occurrence of the first period.

To these two periods a third should be added in the "*Nullpunkt*" of J. Schmulewitsch.[3] Schmulewitsch placed the frog's gastrocnemius in a vessel containing 0.65 per cent. NaCl solution,

over the rim of which the nerve was carried to the stimulating electrodes, so that it was not submitted to the same temperature as the muscle. The muscle was warmed by placing the vessel in which it was fastened in a larger vessel containing water of a constant temperature of from 46° to 47° C., by means of which it could be heated to any given temperature. At a certain temperature, varying with individual muscles from 37° to 41.5° C., the muscle loses its irritability—reaches the "*Null-punkt.*" The irritability could be restored by bringing the muscle to a lower temperature again. The cooling was done in one-half minute by means of fresh cool NaCl solution.

In Marey's results the gradual loss of irritability was associated with heat rigor. But the fact discovered by Schmulewitsch, and shown in this paper, that a complete loss of irritability can be obtained without any heat rigor, thus making the latter quite distinct, makes it necessary to add a fourth period, that of *heat rigor*, to the preceding.

According to Hermann,[4] heat rigor was first observed by Pickford.

Ch. Rouget[6] noticed that the extent of the heat rigor shortening is equal to that of the highest contraction, and he declared them to be of the same nature.

W. Preyer[7] found that after the muscle had gone into rigor its irritability could be regained if, in addition to restoring the circulation, the muscle was bathed in 10 per cent. NaCl or certain other solutions.

Apparatus.

The apparatus I used for warming the muscle consisted essentially of a double-walled brass cylinder, *b* of Plate IV, about 11.5 cms. long and 6 cms. wide. The distance between the outer and inner walls of the cylinder was 1 cm., and through this space warm or cool water was made to pass from a reservoir, *a*, in which the water was always kept some two or three degrees higher than that to which it was desired to have the cylinder heated. In this way the cylinder could be warmed slowly or quickly to any given point and, when desired, its temperature could be kept constant within half a degree for any length of time. The muscle to be warmed was placed within the space

enclosed by the inner cylinder. The cover of the cylinder is of hard rubber, *c*, containing binding screws for the attachment of the wires, and a small hole through which a thermometer can be passed to give the temperature of the interior space in which the muscle lies. In the end opposite to that in which the rubber cover fits is a narrow slot through which the silk thread attached to the tendon of the muscle passes to be fastened to the recording lever *g*, the thread passing over the pulley *e*. The muscle used in all the experiments was the gastrocnemius, and was dissected away from the *os cruris* in the usual way. It was placed horizontally upon an oblong block of wood, enclosed in a bit of the reflected skin, and was kept firmly in position by pinning the *os femoris* and *os cruris* to the wooden block. This method was used whether the muscle was entirely isolated from the rest of the body or whether the whole frog was placed in the warm chamber; and the muscle could easily be arranged so as to keep the circulation entirely uninjured. In one series of experiments the whole frog (curarized) was used, but so arranged that only the leg with the muscle to be experimented upon, with its circulation intact, was within the cylinder, the rest of the animal being placed upon a support just outside of the cylinder. This experiment required, of course, a different cover with a hole of sufficient size to allow the leg to fit snugly into it. The warm chamber was kept moist by means of filter paper saturated with normal NaCl solution, which was placed upon the block of wood beneath the muscle. The contractions of the muscle were registered upon the smoked paper of an ordinary drum kymograph, upon which also a time marker was made to write, recording fiftieths of a second. To prevent the great inertia fling of the muscle lever, a damper was attached, consisting of a fine steel rod attached to the lever and ending below in a disc of parchment paper, with a diameter of about 3.5 cms., which moved in a beaker of olive oil, *f*. For stimulating the muscle, one of the Du Bois Reymond induction coils was used, which was run with a single Daniells cell. For breaking the primary current through the coil, a mercury make-and-break key was employed, and the muscle in all the experiments was stimulated only by the breaking shocks, the making shocks being short-circuited in the usual way by means of a Du Bois Reymond key.

The stimulus was always a single sub-maximal shock, unless otherwise specified. A Centigrade thermometer was used for taking the temperature of the warm chamber.

Experiments.

In the following experiments the muscle was subjected to five different conditions. I have therefore tabulated the results as Series I, II, III, IV, V.

Series I.

In this series only the excised muscles of curarized frogs were used; muscles, therefore, in which there was no circulation of blood and the influence of motor nerves was eliminated. The muscle was prepared and placed in the cylinder and then slowly warmed. Starting from the temperature of the room, 21° to 23°, several contractions were taken at given intervals of temperature, and the muscle was kept at each temperature for about five minutes in order to be certain that the whole muscle had been really heated to that degree.

In this, as in the succeeding series of experiments, the following facts were ascertained whenever possible: 1. The temperature of the greatest contraction; 2. The temperature at which tetanic contractions appeared; 3. The temperature of the loss of irritability; 4. The duration of the loss of irritability; 5. The temperature of heat rigor, and, 6. The time required for the development of heat rigor at a constant temperature.

In general, the contractions show a gradual rise in height to a maximum in the highest simple contraction. At about this temperature contractions of a tetanic nature (see Plate IV, Fig. 3) were obtained in all but No. 2. Then there ensued a gradual diminution in the height of contractions until the irritability was lost. This condition may last for some time, as in No. 18 for 15 minutes, or it may give place at once to heat rigor. The latter develops slowly or quickly according as the temperature is kept at about the point at which the muscle loses its irritability, or is raised somewhat higher. In No. 1 the development of heat rigor required one hour, the temperature being constant at 38.5°. Where in the table the temperature of loss of irritability or of

heat rigor is given at a lower point than that of the highest contraction, the muscle had been previously heated to the highest point given. Plate IV, Fig. 2, gives a typical series of contraction curves, and although representing an experiment of Series II, may be taken as an illustration of the general effects of warmth upon frog's muscle under any one of the five conditions.

SERIES I.

Excised Muscle; Frog Curarized. 7 Experiments.

Number of Experiment.	Temperature of Highest Contraction.	Temperature of Tetanic Contractions.	Temperature of Loss of Irritability.	Duration of Loss of Irritability.	Temperature of Heat Rigor.	Time of Development of Rigor at a Constant Temperature.	Duration of Experiment.
1	36.25°	35.25°–38.25°	39°	....	38.50°	1 hour.	2 hrs. 15 minutes.
2	37.75°	....	37.75°	... .	.....	.....	2 hrs. 35 "
3	36.25°	32.25°–37.75°	39.75°	....	39.75°	.....	1 hr. 47 "
4	33.25°	26.25°–34.25°	34.25°	....	34.25°	.....	1 hr. 45 "
18	38.25°	37.75°–38.25°	37.75°	15 minutes.	36.75°	.....	3 hrs. 7 "
19	38.15°	37.75°–38.75°	38°	....	37.75°	.....	1 hr. 26 "
20	37.75°	36.25°–38.50°	38.25°	....	.....	.....	1 hr. 20 "

In this series the highest contraction was obtained between 36.25° and 38.50°, except in one case, No. 4, which reached this point at 33.25°. No. 20 may be taken as an average experiment for this series, although from 21.75° to 33.50° there is generally a gradual rise in the height of contraction, while in this experiment the curves for this period present no material difference. The maximum contraction is given at 37.75°, while from 36.25° to 38.50° the muscle is in a state of great irritability, as shown by the tetanic nature of the contractions. At 39°, the temperature having reached its highest point, there is a complete loss of irritability. Without trying to keep the muscle in this condition for more than a few minutes, when it was taken from the case and cooled, the irritability was restored when the temperature fell to 33.75°. The contraction at this point is slightly higher than the contraction taken at about the same temperature near the beginning of the experiment. Subsequent warming resulted in diminishing contractions until the irritability was lost a second time at 38°.

Series II.

In this, as in Series I, only the excised muscles were used, but, as the frogs had not been curarized, the nerves were still active. Although the stimulus was sent directly to the muscle, probably the nerves received and distributed it. In this series there were no tetanic contractions.

SERIES II.

Muscle-Nerve. 4 Experiments.

Number of Experiment.	Temperature of Highest Contraction.	Temperature of Tetanic Contractions.	Temperature of Loss of Irritability.	Duration of Loss of Irritability.	Temperature of Heat Rigor.	Time of Development of Rigor at a Constant Temperature.	Duration of Experiment.
5	36.25°	....	36.50°	21 minutes.	36.50°	.....	2 hrs.
6	29.25°	....	36.75°	....	36.75°	.....	1 hr. 18 minutes.
16	37.75°	....	37.75°	....	39.25°	.....	1 hr. 45 "
17	38.25°	....		....	.....	.....	2 hrs.

In No. 6 the second contraction at 29.75° was the highest, and the contractions then diminished as the muscle was warmed. To see if this diminution could be checked, at 36.25° the muscle was again cooled, but the irritability was only slightly restored. In No. 16, although the highest contraction was obtained at 37.75°, there was but a slight loss of irritability until at 39.25°, when heat rigor commenced.

During the first part of the development of rigor, contractions gradually decreasing in height were given. In No. 17 there is a typical series of contractions (see Plate IV, Fig. 2), gradually increasing in height until 38.25°. Then allowing the temperature to fall slightly, and keeping it constant at from 36.25° to 37° for about twenty minutes, the irritability did not remain constant, but steadily fell; even lowering the temperature still further was of no avail, and at 28.25° there was a total loss of irritability.

In all of these experiments there was some development of heat rigor, although very small, while the contractions were diminishing, so that this condition could not be marked off so distinctly as in the other series.

Series III.

In this series the muscle was dissected free from the distal attachment, but was left with the blood-vessels uncut at the proximal end. The whole frog having been curarized, was pinned to the block in such a manner that the circulation was not impaired, and then warmed. If the heart had ceased to beat when the muscle had lost its irritability, then, when the irritability of the muscle was restored by cooling and bathing in normal NaCl, the action of the heart was also generally restored. In these experiments the loss of irritability was somewhat more rapid than in those upon the excised muscles.

SERIES III.

Muscle with Circulation; Whole Frog Warmed; Frog Curarized. 7 Experiments.

Number of Experiment.	Temperature of Highest Contraction.	Temperature of Tetanic Contractions.	Temperature of Loss of Irritability.	Duration of Loss of Irritability.	Temperature of Heat Rigor.	Time of Development of Rigor at a Constant Temperature.	Duration of Experiment.
7	34.25°	....		....	33.75°	....	1 hr. 40 minutes.
8	34.25°	....		.....	36.75°	....	1 hr. 6 "
9	35.75°	....	36.75°	1 hour.	.....	.....	3 hrs. 37 "
10	33.25°	....	37.25°	53 minutes.	39.25°	35 minutes.	4 hrs. 23 "
11	38.75°	37.75°–39.50°	39.25°	.....	39.50°	1 hr. 28 min.	3 hrs. 14 "
12	36.75°	....	37.25°	7 minutes.	.....	.....	3 hrs. 5 "
21	37°	....	37°	36 minutes.	.....	.....	2 hrs. 38 "

In No. 7 the maximum contraction was at 34.75°. Raising the temperature, the irritability decreased, until at 35.75° only the faintest tremor could be obtained. Lowering the temperature to 29.25°, only the slightest irritability was shown, while at 26.75° there was none. Again raising the temperature, heat rigor commenced at 33.75°. Substantially the same results were obtained in No. 8, except that a loss of irritability came when the temperature had fallen to 29.25°, and heat rigor on again warming to 36.75°. In No. 9 the loss of irritability lasted for one hour, the temperature gradually falling. When the frog was taken from the warm chamber and bathed in normal salt solution, the irritability of the muscle was restored.

In No. 10 the loss of irritability was obtained twice—the first time at 37.25°, and then the muscle was allowed to cool off slowly. After 35 minutes, there being no irritability at 31.25°, the frog was taken from the case and cooled still more by bathing in normal NaCl. In 20 minutes at 23.75° the irritability was restored, and the muscle gave its highest contraction in this condition at that temperature. Again warming the frog, the irritability was a second time lost at 36.25°. Holding this temperature constant for 30 minutes, there was neither irritability nor heat rigor, but on increasing the temperature 2°, rigor commenced. Keeping the temperature constant at 39.25°, the development of heat rigor required 35 minutes.

In No. 11 marked tetanic contractions were obtained from 37.75° to 39.50°, but only after a rest of from two to five minutes between them, for stimuli sent in upon the next revolution of the drum after obtaining a tetanic contraction produced only the normal curve (see Plate IV, Fig. 3). In this experiment the development of heat rigor while the temperature remained at from 38.25° to 39.50° required 1 hour 28 minutes.

In No. 21 the highest contraction during the whole experiment was given at 32.25°, the muscle having been previously warmed to 36.25°, where the irritability was lost. The muscle was then kept constant in this condition at 36.25° to 37.25° for 36 minutes, and again the irritability was restored on cooling.

Series IV.

The frog pithed, and not curarized, was placed within the cylinder just as in Series III and gradually warmed. The circulation and the nerves of the muscle were therefore not directly impaired. As in the case of the experiments upon the muscle-nerve preparation, this series is marked by the absence of any tetanic contractions, which phenomenon therefore is no doubt due to the presence of the nerve.

SERIES IV.

Muscle with Circulation; Whole Frog Warmed; Frog Pithed. 3 Experiments.

Number of Experiment.	Temperature of Highest Contraction.	Temperature of Tetanic Contractions.	Temperature of Loss of Irritability.	Duration of Loss of Irritability.	Temperature of Heat Rigor.	Time of Development of Rigor at a Constant Temperature.	Duration of Experiment.
13	33.25°	....	37.75°	21 minutes.		....	2 hrs. 27 minutes.
14	32.75°	....	35.25°	....	.....	....	2 hrs. 25 "
15	36.75°	....	37°	37 minutes.	.....	....	2 hrs. 49 "

In No. 13, when the muscle had lost its irritability at 37.75°, although immediately cooled to 23.75°, only the faintest trace of irritability was restored, and that after 21 minutes; while heat rigor commenced slowly and continued as the muscle was again warmed. In No. 14, after the irritability was once lost, it was not restored. In No. 15 the muscle was kept at a temperature of 36° to 37.75° for 55 minutes before there was a loss of irritability, although heat rigor commenced after 27 minutes at 37.75°. Allowing the temperature to fall 0.75° in the next 28 minutes, heat rigor still continuing to a small extent, the loss of irritability was obtained. This condition was held for 37 minutes, during which time the muscle was cooling off, then the irritability was somewhat restored, but the muscle all the time developed more rigor. Again, as the temperature rose the rigor became more marked, but the muscle still retained its irritability until very near the end of the period.

Series V.

In this series the experiments without doubt were performed under the most favorable conditions. The frog was prepared as in Series III with perfect circulation, but, instead of warming the whole frog, only the leg including the muscle to be experimented upon was warmed.

The remainder of the body, resting upon a small shelf just outside of the cylinder, was kept cool by frequent bathing in normal NaCl solution. The fact that in No. 22 the muscle was

subjected to varying conditions of heat for five hours and twenty-five minutes without any rigor, shows that, although the muscle has lost its irritability and had been kept in this condition for a long time, yet if one is careful not to go beyond this point there is no apparent injury to the muscle from the influence of warmth.

SERIES V.

Muscle Only Warmed; With Circulation; Body Cool; Frog Curarized.
4 Experiments.

Number of Experiment.	Temperature of Highest Contraction.	Temperature of Tetanic Contractions.	Temperature of Loss of Irritability.	Duration of Loss of Irritability.	Temperature of Heat Rigor.	Time of Development of Rigor at a Constant Temperature.	Duration of Experiment.
22	34°	36.5°–36.75°	36.25°	30 minutes.			5 hrs. 25 minutes.
23	38.75°	36.7°–38.75°	39°				3 hrs. 13 "
24	39.25°	38.75°	38.75°				4 hrs. 47 "
25	.....	.....	.....	.....	41.75°	50 minutes.	1 hr. 17 "

In No. 22 the highest contraction was at 34°, but the following contraction at 35.75° was only one-quarter its height. Holding the temperature constant at from 35° to 37.12° for 1 hour 6 minutes, the contractions, taken about every five minutes, were almost exactly equal. Then raising the temperature not more than 1°, the irritability commenced to diminish until it was lost after 30 minutes at 36.25°. The temperature being kept at this high point, the state of loss of irritability remained without any heat rigor for 30 minutes. Then cooling the muscle, the irritability was partially restored immediately. Allowing the muscle to rest for 1 hour 10 minutes, the irritability was completely restored. Again warming it, tetanic contractions were given at 36.5° and 36.75° higher even than those given at 34° during the first part of the experiment. Again, holding the temperature constant at from 35° to 37.75° for 1 hour 45 minutes, the contractions were not quite uniform, there was a constant though slight diminution in the successive contractions until the period ended in complete loss of irritability.

In No. 23 the usual series was given; the highest contraction

at 38.75°, tetanic contractions from 36.37° to 38.75°, and loss of irritability at 39°. This was with a sub-maximal stimulus; but upon increasing the stimulus to maximal, a tetanic contraction higher than any preceding contraction was given. Keeping the temperature constant at from 38.75° to 41.25° for 1 hour 43 minutes, and using a maximal stimulus, the contractions gradually diminished, losing their tetanic nature after 25 minutes until the period ended in loss of irritability, while at the same time during this period the muscle had developed a large amount of its heat rigor. Now pushing the secondary coil completely over the primary, making the stimulus as much *supra-maximal* as possible, an additional small contraction was given, which was somewhat increased when the stimulus was sent in as rapidly interrupted induction shocks. In 26 minutes from the loss of irritability for a *maximal* stimulus even this enormous *supra-maximal* stimulus was unable to call forth a contraction.

Putting the frog, still cool and unharmed, aside in normal NaCl solution for about 14 hours, the irritability had again been partially restored, a maximal stimulus causing a contraction ¼ the height of the highest contraction, the stimulus being equal to that used in the first part of the experiment. Now warming the muscle to a high temperature, only a small curve of heat rigor was obtained. This would represent about one-third, and the curve of rigor obtained in the first portion of the experiment about two-thirds of all the rigor registered. But comparing this whole curve with the heat-rigor curve of the gastrocnemius from the other leg, which curve was considerably higher, shows that after taking the muscle from the cylinder some rigor must have developed which was not registered.

In No. 24, using a *maximal* stimulus from the beginning, a regular series of contractions reaching a maximum at 39.25° was obtained. From this point the temperature was held constant at from 39.25° to 38.75° for 1 hour 40 minutes. In 1 hour 8 minutes heat rigor commenced. At the end of this period the stimulus was made *supra-maximal*, but with scarcely any effect. However, when the muscle was cooled, bathed in normal NaCl and set aside for about 2 hours, the irritability was somewhat restored.

In one experiment upon a frog, not curarized nor pithed, the whole animal being warmed, a regular series of contractions

was obtained reaching a maximum at 36.75°. This highest contraction was also the only one in the whole experiment of a tetanic nature. In addition to these contractions caused by the regular single sub-maximal shocks there was an irregular series of voluntary contractions, increasing in height as the warmth increased until they too attained their maximum at 36.75°. But although the highest contractions, both voluntary and those caused by the electric stimulus, were obtained at the same temperature and about the same time, the former were 2½ times the height of the latter. In this condition of greatest irritability the frog gave a number of convulsive voluntary contractions which entirely ceased in about 5 minutes, while period B (see below) continued as in the other experiments.

Influence of Warmth upon the Irritability of Motor Nerves.

Three experiments were made upon motor nerves, using the same apparatus as in Series V, warming the sciatic nerve and registering its condition by means of the gastrocnemius, which rested upon the support outside, and was frequently bathed in normal NaCl solution.

In the first experiment with a single sub-maximal induction shock, the contractions showed no difference up to about 42.25° after having been warmed for 1 hour 23 minutes; then there was a slight diminution so that 33 minutes later at 48.75° a contraction was only given with rapidly interrupted induction shocks, and this irritability soon disappeared. Then, on allowing the nerve to cool, its irritability was restored. This process of warming to loss of irritability and then restoring was repeated several times with no apparent injury to the nerve.

The second experiment was practically a repetition of the first except that the diminution in height of the contractions commenced at 45.25°, and by holding the temperature constant within 1½° for 12 minutes a loss of irritability was obtained.

In the third, the nerve lost its irritability for a sub-maximal stimulus at 48.25°, and on continuing to warm it to 50° there was still no irritability manifested, until the stimulus was increased by pushing the secondary coil to "6"; then a contraction was

given. Still raising the temperature, the nerve again lost its irritability at 51.25° with the coil at "6," but at 51.75°, by pushing the secondary coil to "4," another contraction was obtained.

On now maintaining the temperature at this point for 4 minutes, irritability was lost for the stimulus "4." On again increasing the stimulus to "3½," a contraction of ⅓ the height of the last was given, but the response to this stimulus soon ceased.

Next, on warming to 53.25°, pushing the coil to "3" and making the stimulus tetanic, only a very small contraction resulted. This soon disappeared, and no further increase of stimulus was able to cause a contraction at this temperature, but as soon as the nerve was cooled the irritability returned, although somewhat impaired.

N. Afanasieff[8] in his paper, "Investigation upon the Influence of Warmth and Cold upon the Irritability of the Motor Nerves of the Frog," has emphasized several facts. His method was to let the nerve rest easily upon two hook-like stimulating electrodes placed within a glass tube. The warming was accomplished by bringing pure oil, heated to the desired temperature, in contact with the nerve. A minimal stimulus was used. The degree of irritability of the nerve was determined by moving the secondary coil. Gradually warming the nerve to 35° C. gave an increase of irritability. Suddenly warming the nerve to 35°–40° caused clonic spasms of the muscle. By warming to 40°–45° convulsions of a tetanic nature were produced, as Rosenthal also had observed. These convulsions may last for a minute. At 65° the irritability is destroyed almost immediately. Between 40° and 50° the irritability is lost, but can be restored upon cooling. This, Rosenthal and, according to Hermann,[9] Pickford also, had observed. A condition which Afanasieff calls the "apparent death of the nerve," wherein it is absolutely unexcitable to the *strongest* stimuli, is obtained at 50°–65° C. But by cooling the nerve the irritability can be restored, though incompletely.

There are several differences between the results of Afanasieff's work and those of this paper, the methods being quite unlike in the two cases. The highest temperature at which I could get a contraction with the strongest stimulus was at 53.25° C., and beyond that the nerve went into "the condition of apparent death."

When the irritability was lost with a sub-maximal stimulus, not only was a contraction given at the same temperature by increasing the stimulus, but also at a somewhat higher temperature. And this process of losing and regaining the irritability was repeated at successively higher temperatures until the limit was reached.

Conclusions.

In general, four periods, representing distinct phases of the condition of irritability, were obtained in each of the five series. These may be described as, *A*, gradual increase of irritability to the maximum; *B*, gradual diminution of irritability to its total loss; *C*, condition of no irritability, and *D*, development of heat rigor. In these separate phases the results of the whole course of experiments may be summarized.

Period A.

Gradual Increase of Irritability to the Maximum.

This in general corresponds to Marey's[2] first period "excitation of muscular action," in which he obtained the highest contractions at from 30° to 35°. But my results differ in making the maximum contractions at from 32.75° to 39.25°, with only one exception, No. 6, Series II, where it was 29.25°, and this no doubt was abnormal. Marey also says that if a muscle be previously submitted to a low temperature and then warmed, the descent of the contraction curve becomes shortened, thus marking the transition between the lengthening of movement by cold and its acceleration by warmth. This is evidently true for this epoch of transition; but from the temperature at which the muscle enters into period A, say 22°–26°, there is generally a slight *increase* in the duration of the contraction, so that the contraction lasts .02 or .03 of a second longer in the condition of greatest irritability than it does at the beginning of period A. In addition to Marey's results, during and near the temperature of greatest irritability, I obtained tetanic contractions, the stimulus being simply a single *sub-maximal* shock. These tetanic contractions were obtained in ten experiments included in Series I, III and V, but not in either Series II or IV, so that

whenever the *uninjured* nerve was present they were absent. As shown in the experiments on nerves, at a period when the muscle is in its condition of greatest irritability the nerve is scarcely affected by heat. Hence, as the nerve probably received and distributed most of the stimulus, this may explain the apparent anomaly. These tetanic contractions were usually more marked after a rest of four or five minutes than in contractions observed after a shorter rest, as one or two minutes, and they sometimes disappeared entirely in the latter case.

Period B.

Gradual Diminution of Irritability to Total Loss.

In Marey's second period, "alteration of the muscle by warmth, and loss of irritability," the phases in periods B and C were united, while period D was not brought out at all.

In period B there is a gradual diminution in both the height and duration of the contraction. If one is very careful to stop the rise of temperature immediately after the highest contraction and at that point keep it constant, this period may last for one or two hours. If, however, the temperature rises only one or two degrees above that at the beginning of the period, the loss of irritability is considerably hastened, and heat rigor may commence. Exercising considerable care, however, this period may be carried into period C without causing any rigor in the muscle.

Period C.

Condition of No Irritability.

This period was pointed out by the investigation of Schmulewitsch,[3] whose methods have been described. Schmulewitsch, however, did not keep the muscle in this condition as I have done, but restored its irritability at once by means of cool 0.65 per cent. NaCl solution. Normal NaCl is not necessary to restore the irritability, although it promotes the restoration. Simple cooling is sufficient.

This period may come at any point within 5° above the temperature of the greatest contraction (in No. 6, Series II, there was a difference of 7°), or it may come at a somewhat lower

degree if, after period B is well begun, the temperature is allowed to fall. I have found that this period may last if the temperature is kept constant, as *e. g.* No. 21, Series III, for 36 minutes or more; while, if the temperature be allowed to fall slowly, as *e. g.* No. 9, Series III, it may last for one hour.

Period D.

Development of Heat Rigor.

After the restoration of the muscle's irritability, the extent of the contractions is generally smaller than that of those of period A, the first contraction being usually the largest; then they diminish until a second loss of irritability. To this, however, there are marked exceptions, in one or the other particular, or in both, as *e. g.* No. 22, Series V, where the conditions of the experiment were more favorable than in the preceding series. This *restored* series may be obtained without any heat rigor, but a loss of irritability usually comes sooner than in period B. Heat rigor commences generally at a temperature from 5° to 1° or 2° above that of loss of irritability; but if in period C the muscle be kept sufficiently long at a constant temperature, no further increase is necessary to produce heat rigor. The time of development of rigor at a constant temperature varies from 35 minutes, as *e. g.* No. 10, Series III, to 1 hour 28 minutes, as *e. g.* No. 11, Series III.

The regular series of long contractures which Marey[2] obtained during the development of heat rigor I was unable to get. Unfortunately, Marey does not give us the exact method by which his curves were obtained. During the first portion of the development of rigor, contractions can be obtained, but they do not show long contractures. However, if a period of rest intervenes, a contraction then taken may give the long contracture; but the curve of the succeeding contraction, taken after one revolution of the registering drum, generally, as in the normal curve, comes at once back to the base line. The long-lasting contracture is exceptional.

To Dr. W. H. Howell, who designed the apparatus (which was made in the workshop of the laboratory), and gave me many valuable suggestions during the course of these experiments, I wish to express my indebtedness and gratitude.

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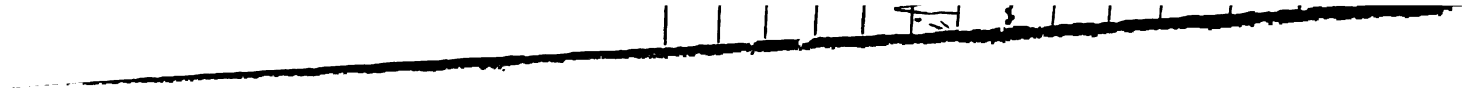
EXPLANATION OF PLATE IV.

FIGURE 1. Apparatus used in warming the frog's muscle (described page 20).

FIGURE 2. Showing the effect of gradual increase of temperature upon the contractions of the muscle. The temperatures indicated by the different letters are as follows:

a,	26.25°	*e*,	37.25°	*i*,	37°	*m*,	31.75°
b,	30.75°	*f*,	36.75°	*j*,	36.25°	*n*,	30.25°
c,	34.75°	*g*,	38.25°	*k*,	36.75°	*o*,	29.25°
d,	36.75°	*h*,	36.25°	*l*,	33.75°	*p*,	28.25°

FIGURE 3. Showing the tetanic contractions obtained with single induction shocks. The temperatures indicated by the letters are as follows: *f*, 37.25° *g*, 37.75°.



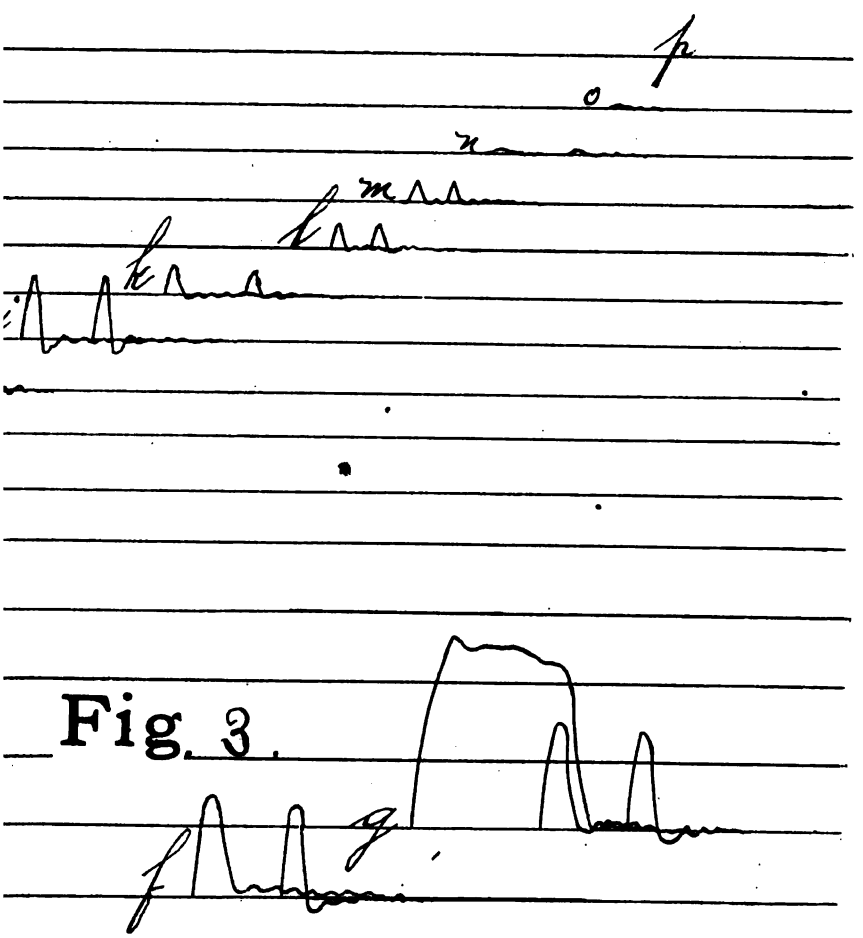


Fig. 3.

EXPERIMENTS IN REGARD TO THE SUPPOSED "SUCTION PUMP" ACTION OF THE MAMMALIAN HEART.

By H. NEWELL MARTIN, D. Sc., M. D., M. A., F. R. S., Professor in the Johns Hopkins University, and FRANK DONALDSON, Jr., B. A., M. D., formerly Graduate Scholar of the same. With Plate V.

That the heart acts not only as a force pump during its systole, but during its diastole actively expands and thus gives rise to a negative pressure, which causes blood to move from the systemic veins to the right auricle, has long been maintained. Indeed, while the thorax and lungs are intact there must undoubtedly be some such "suction" action; the elastic reaction of the lungs pulling on the heart must expand it when its walls are relaxed and flabby in diastole. The heart during its systole does extra work in overcoming pulmonary elasticity (as evidenced by the negative pressure then observable in the lungs[1]), and during diastole gets this work returned to it as a distending force.

But the question still remains whether, after the thoracic cavity has been opened, the heart expands in diastole passively in consequence of the positive pressure exerted by blood driven into it from the veins, or actively, and in the latter case, by causing a negative pressure, causes blood to flow towards itself, even when there is no positive pressure in the venæ cavæ? Fick,[2] nearly forty years ago, showed that the hearts of oxen, sheep and men, when in *rigor mortis*, actively expanded after compression; but he also demonstrated that perfectly fresh excised hearts had no such tendency: they only expanded and received liquid from outside when it was sent into them under positive pressure.

It was, however, conceivable that the heart in the living body, with blood flowing through the coronary arteries and capillaries and veins, might behave differently. If we imagine the relaxed

[1] Voit, Zeitsch. f. Biol. I, p. 390; Klemensiewicz, Mitt. d. Ver. d. Aerzte in Steiermark, 1875–76, p. 41: and others.

[2] Müller's Archiv, 1849, p. 283.

heart as a bag with very soft and distensible walls, it is easy to see that a network of distended elastic tubes ramifying in its substance might cause active expansion of its walls and dilatation of its cavities. Starting from this theory, Thebesius and, independently, many years afterwards Brücke, suggested the doctrine of the "*selbst-steuerung*" of the heart. According to this doctrine, the aortic semilunar valves press close against the walls of the vessel during ventricular systole, and cut off the blood-flow to the coronary arteries; these arteries therefore become comparatively flaccid; but during diastole the valves flap back, and blood rushing into the coronaries distends them, and through them the walls of the heart, in which they ramify.

The "*selbst-steuerung*" doctrine in its fullest sense may now be considered as disproved, many facts quite inconsistent with its truth having been demonstrated.

But, although the belief that the mouths of the coronaries are closed during ventricular systole was no longer tenable, it was still possible that the distended elastic arterial branches ramifying in the myocardium should mechanically tend to stretch the walls and dilate the cavities of the heart. During systole the muscular force would easily overcome this stretching tendency of the tense vascular network; but during diastole it is easily conceivable that the muscle might yield in turn and the heart cavities be actively distended. It was to put this question to the test of experiment that the research described in this paper was undertaken. We wished to ascertain whether, at any period of the heart's beat after the thorax had been opened, a negative pressure existed in the right auricle.

The negative pressure proved by Goltz and Gaule to occur in the ventricles for a brief period at the end of the systole, had already been shown by Moens[1] not to affect the auricles, and therefore to be without effect in making the heart a suction pump so far as the venous system was concerned.

The general plan on which we worked was to open the thorax, maintaining artificial respiration and leaving the lungs and the pulmonary blood-flow intact. Then, to place a long tube in the aorta just beyond its arch, and occlude all the branches leading from the aorta except the coronaries. Also, to close the great

[1] Pflüger's Archiv, XX, p. 517.

systemic veins near the heart, except one, which was in connection with a reservoir containing defibrinated blood. This reservoir was connected with the outflow tube tied into the aorta. Thus the whole systemic circulation was represented by the coronary system and a set of rubber tubes which started from the aorta and returned to the right auricle. The heart and lungs being freely supplied with blood, long retained their vitality, and the heart, as we knew by previous work, could in this manner be kept beating normally and vigorously for hours. Then, by connecting a manometer tube, filled with blood, to the side of the cannula leading to the right auricle, and cutting off all blood-supply to the right heart except from this manometer tube, we could easily ascertain whether the right heart exerted a negative pressure, so as to be able, in common phraseology, to "suck" blood out of the manometer after the level of the blood in it was below the level of the right auricle.

The method of experiment was in essential features that described by one of us[1] four years ago. As it has not yet been given in detail in any journal published in the United States, it may be well to here repeat the description, embodying in it such minor changes in the apparatus as have been made since 1883, and a description of the manometer tube and its connections as specially added for the present research.

Small dogs were used; in the present series of experiments they were always deeply narcotized by the subcutaneous administration of sulphate of morphia before the operation was begun. Then tracheotomy was performed, the external jugular vein exposed on one side of the neck, and sufficient curare solution injected into it to prevent reflex muscular contractions. Artificial respiration, maintained uniformly by a small water engine, was started as soon as the curare began to influence the movements of the respiratory muscles.

The further course of the operation was as follows: The pneumogastro-sympathetic trunks were divided on each side of the neck, a cannula put in the right common carotid, and the left ligated. Next, the first pair of costal cartilages and the bit of sternum connected with them were removed, along with the

[1] H. Newell Martin: Direct influence of gradual variations of temperature upon the rate of beat of the dog's heart. Phil. Trans. Roy. Soc. Pt. II, 1883.

episternum; then the internal mammary arteries were tied on their course from the subclavians to the breast-bone. The skin and other tissues covering sternum and costal cartilages were next removed, and the breast-bone and rib cartilages cut away, back to the diaphragm. Then most of the right side of the thorax was removed, any intercostal arteries which spirted being twisted or pinched. The right subclavian artery was prepared, and ligated where it separated from the carotid. The superior vena cava was cleaned, and two ligatures placed loosely around it, to be used subsequently. The right azygos vein was ligated close to its entry into the superior cava, and a ligature was placed loosely around the inferior cava just above the diaphragm.

Proceeding now to the left side of the chest, the subclavian artery was ligated, and, the left lung being gently held aside, the aorta isolated and cleared near the diaphragm. A ligature was placed loosely around the vessel just beyond its arch, and a strong clamp tightened on it to the distal side of this ligature. An aperture having been made in the thoracic aorta near its posterior end, a cannula, filled with defibrinated blood, was inserted into the vessel, and the aortic clamp being removed, was pushed up to the left end of the aortic arch, where the ligature above-mentioned was tied tightly around it. [These aortic cannulas are made of thin brass tubing, and are kept at hand of several sizes, so that one can always be found which fits tightly into the aorta of the animal, and is closely clasped by the elastic walls of that vessel. The cannula has on its distal end a piece of rubber tubing on which is a clamp, which is screwed tight when the tube is filled with defibrinated blood before its insertion into the artery.] The ligature around the inferior vena cava was next tightened, and a cannula filled with defibrinated blood inserted as quickly as possible into the superior cava and tied in place. This cannula was at once connected with *y*, Pl. V, Fig. 1.

So far all the systemic arteries but the coronaries of the heart had been occluded. One common carotid had a cannula in it, and the other was ligatured; both subclavians were ligated below the point at which they gave off any branch, and the aortic cannula was tied in at a level of the vessel, just beyond

its arch, at which it had given off no bronchial or intercostal branches.[1]

The animal was now transferred as quickly as possible to the warm moist chamber, which, with its contents and accessory parts, we have now to describe. It is represented, semi-diagrammatically, in Pl. V, and is 125 centim. in length, 65 centim. in width, and for the greater part of its length is 65 centim. in height. At one end, however, *E*, its height is 130 centim. It has no bottom, but when in use sits in a shallow iron trough (not represented in the figure) filled with water, and raised on supports which admit of Bunsen burners being placed under it; by their means the air in the chest is kept moist and warm. The roof, sides, and one end are glazed; the end, *B*, *E*, is of wood, and perforated by apertures through which several tubes pass. The object of glazing most of the walls of the chamber is to enable a ready view to be had of what is going on inside it; this is apt to be interfered with by condensation of water on the glass during the course of experiment; this drawback may, however, be nearly entirely obviated by smearing the inside of the glass with glycerine. One side of the warm chamber, that turned towards the observer in Pl. V, Fig. 1, is so arranged as to be readily removed and replaced. In it are also several doors which can be opened or closed without removing the whole side of the chamber. These are not represented in the plate. In the chamber are two Mariotte's flasks, *C* and *D*, each of the capacity of two litres. The flasks are entirely similar in all respects, but are so arranged and connected that, at will, either one can in a moment be made to supply blood to the heart or receive blood from it, and always under a constant or any selected pressure, and without opening the warm chamber.

Let us take the flask *C* for more detailed description. Its neck is closed airtight by a rubber stopper through which four glass tubes pass. Two of these tubes, *c* and *d*, end a short way

[1] Sometimes in young dogs a minute branch is given off from the innominate artery to the thymus. This was sometimes tied, but usually neglected, as it is difficult to get at, the amount of blood drained off by it is trivial, and when both venæ cavæ are tied, cannot get back to the heart. The large thymus in puppies makes the operative part of the experiment more difficult in them, but not to any important degree.

below the stopper, and are open to the exterior when the flask is receiving blood. By means of the rubber tubing *l'*, Fig. 1, *c* is connected with the three-way stopcock *X*, Fig. 2, and beyond it, by *l* with the funnels *r* and *w*. From these funnels the flask is supplied with blood. The tube *d*, with its rubber extension, leads to the stopcock *g*, Fig. 2; it, when open, permits the escape of air when blood enters *C* from either of the funnels.

The two remaining glass tubes, *e* and *f*, reach nearly to the bottom of the flask; they are kept closed when *C* is receiving blood, but are opened when it is supplying blood to the heart. Of the two, *f* reaches about half an inch lower than *e*; it is connected by *k* (Figs. 1 and 2) with the three-way cock *W*. From the cock leads the tube *n*, which re-enters the warm chamber, and is connected, during an experiment, with the superior vena cava by the intervention of *o*, *q* and *y*. It serves to supply the heart with blood.

The tube *e* is connected with the stopcock *u*. Through it air enters the flask *C* when it is sending blood to the heart.

The tubes connected with the flask *D* are in all respects similar to those opening into *C*, *c'* answering to *c*, *d'* to *d*, *e'* to *e* and *f'* to *f*, and so forth. The stopcock *X* is so arranged that when the passage from *c* is open that to *c'* is closed, and *W* so that when the passage from *f'* is open that from *f* is shut.

The flasks are represented as resting on the block *S*, in order to avoid more complication in the figure. In reality they stand on a movable platform, which can be raised or lowered; and before using they are raised until the bottom of each is about twelve centimeters above the level of *y*, which itself is about 40 centimeters above the bottom of the water trough on which the warm chamber rests.

Before an experiment the flask *C* is nearly filled with defibrinated blood, and *D* about one-fourth filled. This is done from the funnel *r* by opening and closing the proper passages or stopcocks.

The next step is to fill the tube *f* and its fellow, and all the passages connecting them to *y*, with blood from the flasks. These tubes have alternately, during the experiment, to act as syphons, supplying the heart. To fill them, *X*, Fig. 2, is so turned as to close *l'*, and of course *c*, to which it leads; *g* is

turned so as to cut off *d* from communication with the air; *W* is turned so as to open a free passage from *f* through *k* to *n* and thence through *o* and *q* to *y*. Also, *u* is opened so as to place *e* in free communication with the atmosphere. Suction is then applied to *y*, the stopcock *q* being open. Air then enters *C* through *e*, and blood passes out to *y* through *f*, expelling the air previously in the connecting tubes between *f* and *y*. As soon as all the air is expelled the stopcock *q* is shut. *C* is now a Mariotte's flask; on opening *q* it will deliver blood in an even flow and under uniform pressure at *y*, the flask having of course been previously raised so that the lower end of *f* is higher than *y*.

When the tubes connecting *C* with *y* are full, the stopcocks *X* and *W* are reversed; *g* is opened, *u* closed; and *g'* closed and *u'* opened. Then, on *q* being opened and suction again applied at *y*, the tubes leading from *D* to *y* are filled with blood, so that *D* shall act as a Mariotte's flask. Then *q* is closed, *g'* opened and *u'* shut.

The warm chamber is now closed; the stopcocks *X* and *W* again reversed, so that the fuller fiask *C* shall be in connection with *y*. But *g* is left open, so that as the blood and air in the flask expand while they heat, air may escape. From time to time *g* is closed and *q* opened; a few centimeters of blood are collected in a beaker and the temperature of the blood noted. This temperature is indicated by the thermometer *p*, the bulb of which reaches into the enlargement *o*. The blood collected is returned to *D* by pouring it into the funnel *r*.

Meanwhile, the preliminary operation on the dog was carried on up to the point of opening the thorax. As soon as the temperature of the blood reached about 38° C., the gas flame under the warm chamber was turned low and the operation on the animal completed, as already described. Then the dog was immediately transferred to the warm chamber, and the cannula in its superior cava connected with *y*. At the same moment *g* was closed so that *C* should act as a Mariotte's flask; then *q* was opened and blood allowed to flow into the right auricle of the heart, under a constant pressure equal to about that of a column of blood ten centimeters in height. The clamp on the rubber tube of the aortic cannula was loosened and the heart allowed to pump out blood in a free stream, but still not so free

but that considerable arterial pressure was maintained in the aortic stump and, from it, in the coronary arteries of the heart. After a minute or so, when it was judged that all the blood previously contained in the isolated heart and lungs had been washed out and replaced by defibrinated blood, the rubber tubing on the end of the aortic cannula was quickly slipped over *h* and the clamp on this tubing removed. The heart then pumped blood up through the tube *t* to the funnel *x*, and from the funnel the blood returned by *l* and its side branch *l''* to *D*. Thus as *C* emptied, *D* filled. When *C* was nearly empty and *D* nearly full, it was easy, by reversing the stopcocks *X* and *W* and opening or closing *g* or *g'* and *u* or *u'*, as the case might be, to make *D* the supplying flask and *C* the recipient. Thus the same blood could be circulated again and again through the heart and lungs, and always be supplied from the superior cava uniformly to the right auricle, while aortic pressure could be kept constant; or, if desired, varied within wide limits by raising or lowering the level of the funnel *x*.

Finally, the cannula in the right carotid having been connected with a mercury manometer, which recorded arterial pressure and the pulse rate on the paper of the kymographion, the front of the warm chamber was replaced.

Of course, artificial respiration was maintained throughout the experiment. The air-supply tube from the respiration pump leads to a three-way stopcock; one branch of the stopcock opens into a tube which leads to the operating table; the other branch into a tube which leads into the warm chamber. As soon as the animal is removed from the table to the chamber, the stopcock is turned so that the air blast enters the tube leading to the warm chamber, and this is immediately connected with the cannula in the trachea.

By means of the thermometer *p* the temperature of the blood supplied to the heart can be ascertained; this can be kept fairly uniform by raising or lowering the flames of the Bunsen burners beneath the warm chamber, as desirable. We also found it useful to surround with raw cotton the parts of the tubes *t* and *l* which lie outside of the chamber. By surrounding *C* and *D* with water jackets[1] the blood supplied to the heart

[1] Phil. Trans. Roy. Soc. Lond., Pt. II, 1883.

can be kept at an almost uniform temperature. But as such uniformity was not necessary in regard to the experiments to be described in the present paper, the water jackets were not used. The blood was in every instance kept near 38° C., and never varied more than 2° C. from that temperature during the time in which our observations were made.

It still remains to describe that part of the apparatus, viz. *abm*, which was specially devised for the series of experiments to be described in this paper. Between *q* and *y* is intercalated the top of a T-piece; on the leg of this T-piece is the stopcock *a*. When this is closed, all blood passing *q* must take its course to the heart through *y*; but if *a* be opened, some of the blood will flow into the U-tube *bm*, which is fixed at such a level, after the animal has been put in the warm chamber, that the upper end of the open limb *m* is above the level of the right auricle of the heart. The limb *b* can be opened or closed at will by the stopcock *s*.

So soon as the heart was ascertained to be pumping blood round well and uniformly, *a* and *s* were partly opened and a portion of the blood-supply diverted until the U-tube was filled to near the top of *m*. Then the stopcocks were closed, and all the blood again flowed through the heart. Next, the height of the point of entry of the superior cava into the right auricle was carefully measured from the bottom of the warm chamber. This was done by placing the lower edge of one end of a spirit-level on the cannula connected with *y*, and close to the heart. After getting the level perfectly horizontal, the distance of its lower edge from the bottom of the trough was measured. This gave the height above the bottom of the trough of the upper side of superior cava where it joined the auricle.

If now *q* be closed and *a* opened, the right heart no longer can get blood from the Mariotte's flask. Its only source of supply is from the previously filled U-piece. If it takes blood from this until the level of the liquid in *m* falls below the level at which the vena cava enters the auricle, then the right heart has exerted some suction, it has actively expanded; while if it only continues to supply itself so long as the level of the blood in *m* is higher than the level of the entrance to the auricle, it is clear that the heart only takes blood when supplied to it under some

positive pressure, and that it is not a suction pump. So soon as the level of the blood in *m* ceased to fall, its height from the floor of the trough was measured and noted. Then *q* was opened, and the heart thus again is supplied with blood from one of the Mariotte's flasks, while the U-tube *bm* was also refilled. Then *a* was closed. After waiting a minute or two, *q* was once more closed and *a* opened, and the observation repeated; and so on as often as might be desired, or until the heart began to show symptoms of weakening.

We made in all nine experiments as to the capacity of the right heart to actively take blood from the superior cava after the thorax had been opened; and in the course of each experiment the conditions were varied. At the commencement arterial pressure was medium (about 95 mm. Hg), and the pericardium was intact. Then arterial pressure was varied within wide limits, by raising or lowering the level at which *t* emptied into *x*. Moderate and violent artificial inflation of the lungs were employed, and also the entire cessation of the artificial respiration. Finally, in order to give the heart the greatest possible freedom, the pericardium was cut away, and the observations repeated. But in no single case did we find that the right auricle would receive blood unless that blood were supplied to it under positive pressure, the minimum pressure required varying little from that exerted by a column of blood about 12 mm. in height. It might fall to 8 mm. or rise to 18 mm., but those were the limits.

The earlier experiments were made with the defibrinated blood of young sucking calves. Though the isolated heart of the dog will work well for hours when supplied with this blood, it was possible that it might injuriously affect the elasticity of the heart muscle, so our final experiments were made with the hearts of dogs supplied with defibrinated dog's blood, obtained by bleeding several large animals immediately before the experiment. We append the protocols of two such experiments; as all the others agreed with them in every essential, it is not worth while to print them in detail.

The outcome of our work is this: that once the "aspiration of the thorax" has been eliminated, the right auricle of the mammalian heart will not receive blood unless supplied to it under a decided, if small, positive pressure. While the heart in the

closed thoracic cavity may, and probably does, act as a suction pump, this is not due directly to an active expanding force of the heart, but is the secondary result of the pneumatic conditions prevailing within the normal closed chest cavity. Any cause diminishing thoracic aspiration must therefore greatly hinder the work of the heart; and it is probably more in this manner that the circulation is impeded in certain cases of hydro- or pneumothorax than by direct pressure exerted on the heart itself.

In the experiment protocols appended there are six columns. Column I gives the time; column II, the height of the upper side of the vena cava superior from the floor on which the warm chamber rested; column III, the lowest height of the blood in the manometer tube *abm*, Plate V, Fig. 1, at which the right auricle would receive blood, this height being measured from the floor of the warm chamber; column IV, the differences between the heights recorded in columns II and III, that is, the lowest pressure under which the heart would receive blood from the systemic veins; column V gives arterial pressure as measured by the manometer connected with the right common carotid; in column VI are given notes when necessary.

EXPERIMENT OF DECEMBER 2, 1886.

Dog under morphia and then curare.—Defibrinated dog's blood used.

I.	II.	III.	IV.	V.	VI.
Time. P. M.	Height of upper side of vena cava superior from floor of warm chamber, in millimeters.	Lowest height at which right auricle would take blood, in millimeters.	Minimum positive pressure necessary in order to make right auricle take blood, in millimeters of positive blood pressure in the vein.	Arterial pressure in right carotid, in mm. of mercury.	NOTES.
h. m.					
3.35	412	435	23	130	
3.38	"	447	35	164	Respiration moderate and uniform.
3.42	"	430	18	103	
3.44	"	427	15	80	
3.45	"	432	20	105	
3.46	"	435	23	142	
3.48	"	450	38	183	
3.53	"	432	20	130	The apparatus for artificial respiration was readjusted at 3.54 so as to make the respiratory movements of the lungs violent, both in expiration and inspiration.
3.55	"	462	50	130	
3.56	"	432	20	130	Artificial respiration stopped at 3h. 55′ 50″
3.59	"	432	20	130	Arterial pressure again was varied, while respiration was kept constant and uniform.
4.01	"	445	33	172	
4.03	"	455	43	200	
4.05	"	442	30	135	
4.06	"	440	28	118	
4.07	"	430	18	75	
4.08	"	442	30	125	
4.11	"	452	40	183	
4.13	"	512	100	205	This arterial pressure was too much for the heart to overcome. It began to dilate, and the experiment was ended.

STUD

EXPERIMENT OF DECEMBER 3, 1886.

Dog under morphia and then curare.—Defibrinated dog's blood used.—Respiration varied both in force and frequency during the experiment, and at other times kept constant while arterial pressure was varied.

I.	II.	III.	IV.	V.	VI.
Time. P. M.	Height above floor of warm chamber of upper side of vena cava superior, in millimeters.	Lowest height at which right auricle would take blood, in millimeters.	Minimum positive pressure necessary in order to make right heart take blood, in millimeters of positive blood pressure in the vein.	Arterial pressure in right carotid, in millimeters of mercury.	NOTES.
h. m. 3.44	410	437	27	125	Artificial respiration 24 per minute and moderate.
3.46	"	437	27	125	Respiration stopped.
3.48	"	430	20	125	Respiration violent.
3.50	"	435	25	115	Respiration moderate, and 24 per minute from this time on.
3.52	"	435	25	85	
3.54	"	432	22	110	
3.56	"	440	30	130	
3.58	"	455	45	150	
4.02	"	480	70	170	Heart begins to dilate.
4.05	"	507	97	182	
4.08	"	455	45	125	
4.10	"	440	30	97	
4.12	"	440	30	75	
4.14	"	442	32	130	
4.15					Pericardium cut away.
4.16	"	432	22	130	
4.18	"	435	25	145	
4.20	"	437	27	165	
4.22	"	445	35	200	
4.24	"	440	30	160	
4.26	"	435	25	135	
4.28	"	432	22	100	
4.30	"	432	22	90	
4.32	"	438	28	135	
4.34	"	445	35	175	Experiment ended; heart and lungs being still in very good working condition.

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A NEW DOG-HOLDING APPARATUS. By CHARLES DAWSON, Janitor, Biological Laboratory, Johns Hopkins University. With Plate VI.

The dog-holding apparatus described here is the result of the desire for an apparatus which would fit equally well dogs of very different sizes, and which could be more easily and quickly applied than the other forms which had been in use here.

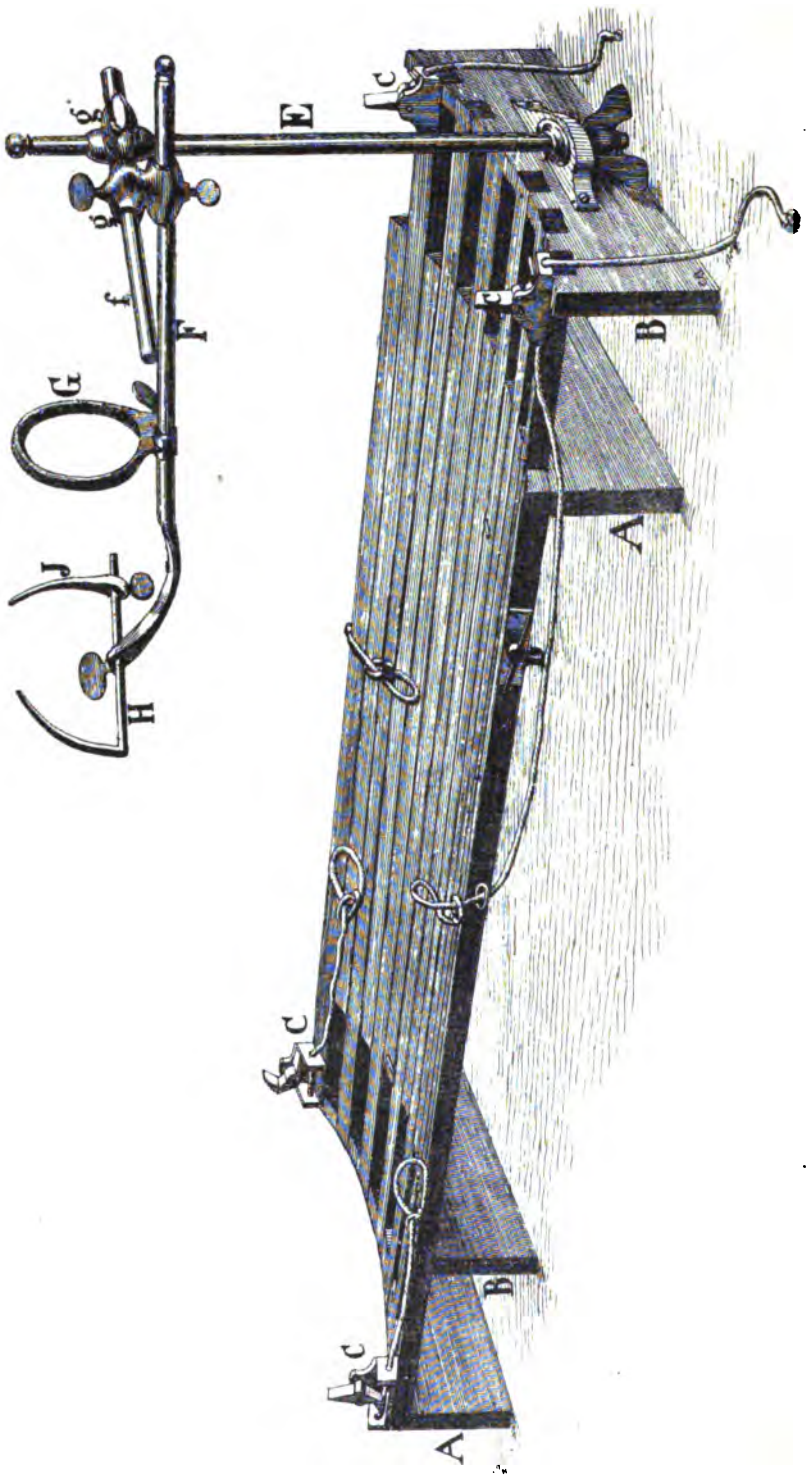
The table or trough is made in the following manner. Two slatted troughs, each sixty centimeters long, are made of some hard wood. One trough has seven and the other has eight slats. The distance between each slat must be equal to the width of a slat, which should be about twenty-five mm. The two troughs are locked together by passing the slats of one between those of the other, and screwing the ends of each on to a piece of board the shape and size of the two boards at the other ends. These four boards serve as the legs of the troughs, which can now be pushed together or drawn out (see Plate), but not separated. They may be clamped at any desirable length by means of a suitable screw, made to pass through a brass plate which is let in from the outside of the larger trough, and buries its point into the adjoining slat.

The legs of the animal are held by passing the cords through brass clamps, made after the manner of those sometimes used for holding the "sheets" on board a yacht. Each clamp consists of two uprights about forty mm. high on a base seventy mm. long. The base is provided with lugs to allow it to be screwed down on the trough. Between the two uprights, which are about thirteen mm. apart, a semicircular foot is riveted through some point, out of center. This foot swings loosely, and clamps the cord when pulled in one direction, but allows it to run free when pulled in the opposite direction. Those figured in the plate are of another style, are more easily made, and answer as well. Four of these clamps are used, and cleats are placed at suitable

points on the troughs, around which the cords may be passed when the position of the legs is to be changed.

A special feature of the head-holder is that it is in no way fastened among or bears upon the teeth of the animal, and on account of this a smaller size is especially applicable to rabbits. It is a modification of one for holding rats which I saw figured in a catalogue and credited to Professor Marey. A steel upright, provided with a collar, washer and nut for clamping it to the trough, is made. A piece of steel thirty-seven mm. long is now taken and flattened at one end, where an enlargement is made. Through this enlargement a square hole is punched, and a thumb-screw is let in at the end. The flattened end of the bar is now bent to suit the curvature of the back of the dog's head. An L-shaped piece of square steel is now made, the upright of the L being thirteen centimeters long and straight, while the base of the L is nine centimeters long and is bent inward and forward. The L can now be run through the square hole made for it, and clamped at the proper point by means of the thumb-screw. Another piece (J, Plate VI), like the base of the L, but having a square hole in one end, provided with a clamping screw, is made, and slides on the long leg of the L. The prongs of the fork thus formed pass down the sides of the back of the head and catch behind the mastoid processes. An oval ring *G*, sixty-five mm. long and fifty mm. wide, with an enlargement on one end, is made. A hole is bored through the enlargement and a thumb-screw is let in. This ring, when in use, is passed along the handle of the fork and clamped when it has passed as far as it can over and around the dog's muzzle. Two brass clamps, with two holes in each bored at right angles and provided with thumb-screws, are made. One of these clamps is put on the upright and the other is put on the handle of the fork. When the holder has been placed on the head of the animal, a bar of suitable size is run through the remaining holes in the clamps and is held in place by the screws. This arrangement of the clamps and bar gives universal movement, and allows the head of the animal to assume an easy position.

The apparatus can be made on a smaller scale for other animals. One for rabbits and cats has been in use here for some time and has given satisfaction.



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EXPLANATION OF PLATE VI.

A, *A* are the boards on which the ends of the trough with seven slats are fastened.

B, *B* serve the same purpose for the inside trough.

C, *C*, *C*, *C* are the four automatic clamps for the cords which hold the legs of the animal.

D is the plate fastened on the outside slat, with a screw passing through it, and binding the two troughs together when they are drawn out for use.

E, the upright of the head-holder.

F, the handle of the so-called fork, flattened and curved at one end to fit the top of the animal's head.

f, *g*, *g'*, the clamping arrangement which gives universal movement.

G, the ring which passes over and around the mouth of the animal.

H is the L-shaped piece.

I, the piece which slides on *H*, both sides moving towards each other till contact with the sides of the neck of the animal is made.

When the apparatus is in use the head-holder would be nearer the base of its upright, and would rest at an angle of about 30′.

The animal is supposed lying on its back.

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NOTES ON THE FAUNA OF BEAUFORT, NORTH CAROLINA.

I.

NOTES ON ACTINIÆ OBTAINED AT BEAUFORT, N. C.

By J. Playfair McMurrich, M. A., Ph. D., Professor of Biology, Haverford College, Pa.

The Actiniæ obtained at Beaufort, N. C., belong altogether to the Littoral Zone, and are, with a single exception, forms whose habitat is sandy or muddy bottoms, or which live upon shells found on such bottoms. This is, of course, due to the conformation of the coast, it being at this point sandy, or, in the "slues," more or less muddy, the only footholds for rock-loving forms being furnished by the Government breakwaters at Fort Macon and on the Shackleford Banks, formed by the deposition of large, irregular blocks of stone brought from a distance, and which are for the most part of too recent construction to have permitted the colonization of many forms.

In the following pages attention will be paid only to the external characters and synonymy of the forms obtained, many details as to structure and variation being withheld for publication at a later date. Much confusion exists as to the synonymy of North American Actiniæ, owing partly to imperfect description, and partly, no doubt, to the want of facilities for their comparison with European forms. I have endeavored to unravel the tangle as much as possible, adopting the generic appellations as defined by Andres in his most admirable and complete monograph of the Actiniæ,[1] this work being the latest and most comprehensive on the subject. We possess already, from the pen of Professor Verrill, to whom we owe so much of our knowledge of North American Actiniæ, a paper[2] on the Actiniæ of North

[1] Andres, A. Fauna und Flora des Golfes von Neapel. IX Monogr.: Die Actinien. Leipzig, 1884.

[2] Verrill, A. E. Brief Contributions to Zoology from the Museum of Yale College. No. XXII. On Radiata from the Coast of North Carolina. Amer. Jour. of Science, 3d Ser., III, 1872.

Carolina, the descriptions in which are from drawings and alcoholic specimens collected by Dr. Yarrow at Fort Macon, and therefore from the same locality that I had the opportunity of studying. This paper would therefore be unnecessary, were it not for the reasons given above, and for the fact that two new forms have been added to those previously known. In Verrill's paper, seven species are mentioned or described, so that, with the two I now add, the number of Actiniæ known to occur at Beaufort is nine. These are distributed in eight genera and four families, the family *Sagartidæ* being the richest, including a majority of all the species.

Fam. SAGARTIDÆ.

1. Cylista leucolena (Verr.), Andres. This form was originally described by Verrill,[1] under the title *Sagartia leucolena*, but has been referred by Andres to the genus *Cylista*, on account of the presence of vertical rows of cinclidal tubercles on the upper portion of the column. I did not observe the peculiarity Verrill describes, consisting in the much greater length and unique coloration of *one* of the gonidial tentacles (*i. e.* one of those opposite the angles of the mouth), but in all the forms observed *both* the gonidial tentacles were longer than any of the others, and were further *both* distinguished by the greater width and distinctness of the white streaks passing from the mouth to them across the disc, when compared with the similar streaks which pass, radiating from the mouth, to the other tentacles of the first cycle. With regard to the odd tentacle, Verrill says that it "is not constantly extended, and is ordinarily not very apparent," and it is possible that the greater elongation was a temporary lengthening of the tentacle for some purpose. The tentacles were arranged in five cycles, their formulæ being 6, 6, 12, 24, 48; all the specimens obtained were small, compared with the measurements given by Verrill, the largest measuring only .5 mm. in length, the diameter of the column and disc, as well as the length of the tentacles, being about the same.

A number of specimens were obtained on loose shells in shallow water at Fort Macon.

[1] Verrill, A. E. On the Polyps and Echinoderms of New England, with Descriptions of New Species. Proc. Bost. Soc. Nat. Hist. X, 1866.

2. ADAMSIA SOL (Verrill). About 1867 Verrill described,[1] from manuscript notes and drawings of Louis Agassiz, an Actinian which had been named by Agassiz *Cereus sol*, and had been collected by him at Charleston, S. C. The coloration of this is practically the same as that of *Adamsia sol*, but the external structural characters are apparently those of an entirely different genus. Thus it is said to have "well-marked suckers" on the column for a short distance below the tentacles, the tentacles themselves are said to amount to "several hundreds" in large specimens, the acontia are extruded through scattered openings, apparently not very numerous, and no mention is made of the conspicuous cinclidal tubercles a short distance above the limbus. It seems strange that a coloration so characteristic and peculiar as that of *Adamsia sol* should be so exactly repeated in an entirely different genus, when there can be no question of protective mimicry either of one another or of a common object, and one would be inclined to suppose that, in some way, a mistake had been made whereby in Verrill's description there has been a confounding of two perfectly distinct forms, the structural characters of one and the coloration of the other having been described. This supposition is indirectly confirmed by Verrill himself in his paper on the Actiniæ of North Carolina, where he describes what is evidently the form I propose here to call *Adamsia sol* under the name *Calliactis sol*, giving as one of its synonyms *Cereus sol* (pars). What Verrill did in this paper was simply to separate from the original compound species that form which served as a type for the color-description, and to describe for it its proper external structural characteristics. The generic term *Calliactis* is evidently a synonym for *Adamsia*, and the latter name having the priority, I have used it here. Andres, not having had opportunities of studying our American Actiniæ, takes the original confused description as correct, and refers this mythical species, composed partly of one form and partly of another, to the genus *Heliactis*. No doubt the structural characters of the compound *Cereus sol* correspond more closely with those of the genus *Heliactis* than

[1] Verrill, A. E. Revision of the Polypi of the Eastern Coast of the United States. Mem. Bost. Soc. Nat. Hist. I, 1866–69.

with those of any other genus, and the question arises, What is the *Heliactis* which Agassiz found? In Verrill's description, as a kind of appendix to the more detailed account of the *Cereus*, a brief description is given of a form named by Agassiz *Actinia guttata*, and supposed by him at one time to be a young stage of *Cereus sol*. Is it the *Heliactis?* I did not find at Beaufort any form I could homologize with the *Actinia guttata;* it would be interesting to ascertain if such a form can now be obtained in Charleston Harbor, where Agassiz first found it, and to determine exactly what are its relationships. Whether Agassiz or Verrill is responsible for the confusion of the first description, I cannot state; but however that may be, the term *Cereus sol* must be considered a *nomen delendum*, and to Verrill, for his description of *Calliactis sol*, must be given the honor of having first described the species. It is not improbable, however, that it may be identical with that described by Lesueur[1] as *Actinia tricolor* and referred by Milne-Edwards and Haime[2] to the genus *Adamsia*, in which case this name would have the priority.

To Verrill's description[3] the following points may be added: The upper circle of brownish-purple cinclidal tubercles is complete, and below it is usually a second circle of smaller tubercles more or less complete. Occasionally a single row only is present, and in some individuals the tubercles of both circles are of the same size, small, and more numerous than usual. Tentacles are arranged in five cycles, their formulæ being 12, 12, 24, 48 and 96, and they are distinctly entacmæous. Acontia are readily emitted from the cinclidal tubercles and from the mouth. As regards the coloration, the column is some shade of yellowish-brown marked with pale brown to dark purplish-brown blotches, arranged more or less distinctly into longitudinal bands. The lips and pharnyx are bright canary-yellow, and outside this is a circle of rich crimson, varying in some specimens towards purple. These two particulars of the coloration are very characteristic, since they remained constant in forms which presented consider-

[1] Lesueur, C. A. Observations on Several Species of the Genus Actinia, illustrated by Figures. Jour. Acad. Nat. Sci. I. Philad., 1817.

[2] Milne-Edwards and Haime. Hist. naturelle des Coralliaires proprement dites. Paris, 1857.

[3] *Loc. cit.*

able variation in other points. The tentacles are reddish or yellowish-brown, or much lighter, ringed with brown, or occasionally unmarked. In some individuals the gonidial tentacles were bright orange-red, in which case the acontia, usually white, were also orange-red.

Specimens of the average size had dimensions as follows: Length of column 2.7 cm.; diameter of column 1.3 cm.; diameter of base 1.8 cm.; diameter of disc 1.4 cm.; length of inner tentacles .7 cm.; length of outer tentacles .3 cm. They live upon shells, usually those of *Neverita duplicata*, inhabited by hermit-crabs.

3. AIPTASIA PALLIDA (Agass.). This form was first described by Verrill from Agassiz's manuscripts as *Dysactis pallida*,[1] but later on[2] he made this same form the type of a new genus, which he termed *Paranthea*, so that in his list of North Carolina Actinians we find it mentioned as *Paranthea pallida*. Andres refers it to its proper genus, but changes unnecessarily the specific appellation, calling it *Aiptasia Agassizii*. I prefer to retain the original specific term, agreeing with Andres, however, as to the generic.

To Verrill's description the following points may be added: The cinclidal tubercles are about the middle of the column, arranged in one or two circles, the lower circle being frequently only partly developed, its tubercles being placed vertically below those of the upper circle, which are twelve in number. The tentacles are very strongly entacmæous, and are arranged in four cycles, their formulæ being 12, 12, 24 and 48. The length of the inner tentacles is about 2 cm., that of the outer ones about .6 cm.

As to coloration, it is possible to distinguish two varieties. The form described by Verrill was apparently very light in color, and hence the specific name. Some specimens found at Beaufort were also very light; but in the majority the general color was a dark chestnut-brown. This form we may call *Variety α*, and its description is as follows: Ground-color of the column is pale brown, almost concealed by numerous chestnut-brown longitudinal bands arranged in regular groups. Each group consists of a

[1] Mem. Bost. Soc. Nat. Hist. I, 1866–69.

[2] Verrill, A. E. Synopsis of the Polyps and Corals of the N. Pacific Exploring Expedition, etc. Proc. Essex Inst. V.

median broad band bearing a pair of cinclidal tubercles (*i. e.* a tubercle of each circle), and on either side of this, and separated from it by a narrow portion of the ground-color, are two narrower bands. Each of these groups, there being twelve in all, is separated from the succeeding one by a very fine dark-brown line, dividing what would be otherwise a comparatively wide band of the ground-color. The cinclidal tubercles are of the ground-color. Tentacles are brown, spotted with white, and usually with one or two distinct transverse white bands; the outer tentacles have no white markings. Disc chestnut-brown, shading off to a greater transparency towards the bases of the tentacles. Lips and acontia white.

The other variety, which, since it was the one originally described, must be considered the type, has the column almost white in its lower two-thirds, becoming brownish above, and being there marked with longitudinal bands arranged as in *Var. α*. The markings are the same as those of the variety, but are more indistinct throughout.

This form, or rather the *var. α*, is the most common form at Beaufort, occurring on the wharves, on shells on the mud-flats, and in very large numbers on the oyster-rocks in the "slues" off the eastern end of Bird Shoal.

4. SAGARTIA PUSTULATA, *nov. sp.* Base ample, wider than the column, and strongly adherent. Limbus crenate. Column low, with deep longitudinal sulci and transverse rugæ, so that it appears almost as if tuberculate. Margin indistinct. Tentacles long, slender, entacmæous, arranged in four cycles, their formulæ being 8, 8, 16 and 32. Disc moderate, concave. Peristome lobed, prominent. Acontia emitted with reluctance from the mouth and upper portion of the column.

In color the column is green, darker above, and verging in some specimens to olive-green. Tentacles are pale olive-green, with scattered yellowish-white, opaque spots. Disc is of the same shade of green as the upper portion of the column, and is flaked with opaque yellowish-white spots, which arrange themselves more or less regularly in bands and spots, the bands occurring at the bases of the tentacles. At the bases of each of the tentacles of the first and second cycles, but especially noticeable in the former, is a vermilion patch.

The height of the column is about .6 cm.; diameter of the base 1–1.4 cm.; length of tentacles of the inner cycle .9 cm.; diameter of the disc .6 cm. It was found on shells in shallow water at Fort Macon.

5. SAGARTIA GRACILLIMA, *nov. sp.* Base adherent. Column slender, extensible, with longitudinal sulci, and when not fully expanded somewhat rugose; in partial contraction the upper portion of column folds into the lower, as Verrill has described for *Cylista leucolena*. Tentacles short, slender, entacmæous, arranged in four cycles, their formulæ being 6, 6, 12, 24. Lips prominent and lobed. Acontia emitted with reluctance from the mouth, at the upper part of the column, and at the limbus.

The column is yellowish, transparent, the upper portion appearing orange from the internal parts shining through. Disc with numerous opaque yellowish spots, aggregated into an irregular ring around the mouth, and some lines radiating out towards the bases of the tentacles. Tentacles transparent, with a few opaque yellowish-white spots similar to those of the disc. Peristome olivaceous. Pharynx orange. Acontia white.

Length of column when partly expanded about .7–.8 cm., when fully expanded 2 cm.; breadth of column when partly expanded about .5 cm., when fully expanded .3–.4 cm.; diameter of disc about .3–.4 cm.; length of tentacles about .2 cm.

Obtained two specimens only; one was found on the bottom of a barge, in company with sponges and hydroids, the other on a loose shell. The specific name *gracillima* is bestowed as signifying the extreme slenderness of the column when in full extension.

This and the preceding species both present the features of the genus *Sagartia* as seen in the presence of acontia, the tentaculate margin, the absence of any folds or tubercles upon the column, the presence of cinclides scattered irregularly over the column, but most numerous in its upper half, and the comparatively small number of tentacles, and they have, therefore, been referred to that genus.

FAM. BUNODIDÆ.

6. PHYMACTIS CAVERNATA (Bosc), Andres. According to Andres, this form was first described by Lamouroux as *Actinia*

cavernata of Bosc. The first mention of it by an American author was by MacCrady,[1] who described in 1859 the fission of a form which he termed *A. cavernosa.* In Verrill's revision of the "Polyps of the East Coast" it is described as *Bunodes cavernata;* but later that author, when establishing the genus *Cladactis* for certain species obtained on the Pacific Coast,[2] referred this form to it; and it is mentioned under the name *Cladactis cavernata* in the same author's account of the Actiniæ of North Carolina. Andres, in his monograph, refers it to the genus *Phymactis,* but states in a note his uncertainty whether it should not be retained in the genus *Bunodes.* I have no doubt that it is a *Phymactis.* The irregularity in the arrangement of the tubercles in the adult, although in very young forms they are arranged in vertical rows, is very evident. The acrorhagi of the margin are in two rows, and of a different color than the tubercles of the column. The tentacles are arranged in four cycles, their formulæ being 12, 12, 24 and 48.

This form is quite abundant at Fort Macon, where it occurs attached to the stones of the old Government breakwater above low-water mark.

7. AULACTINIA CAPITATA, Agass. This handsome white form retains the name given to it by Agassiz in the manuscripts used by Verrill in compiling his revision. The description there given is tolerably correct, and it need merely be added here that the tentacles are in four cycles, their formulæ being 12, 12, 24 and 48.

A few specimens were obtained buried to the tentacles in the sand on Bird Shoal.

FAM. PARACTIDÆ.

8. PARACTIS RAPIFORMIS (Lesueur), M.-Edw. Was discovered by Lesueur in 1817,[3] and named *Actinia rapiformis,* but afterwards was referred to the genus *Paractis* by Milne-Edwards and Haime.[4] Verrill, in all his papers prior to 1872, retains the

[1] MacCrady. Proc. Elliot Soc. Nat. Hist. I. Charleston, 1859.

[2] Verrill, A. E. Review of the Corals and Polyps of the West Coast of America. Trans. Conn. Acad. I, 1866–71.

[3] Lesueur. Journ. Acad. Nat. Sci. I. Philad., 1817.

[4] *Loc. cit.*

generic term *Actinia*, but in his list of North Carolinian Actiniæ adopts Milne-Edwards' name.

A single specimen was obtained which probably belonged to this species, but unfortunately it died before a proper study of it had been made. This is all the more unfortunate since very little is accurately known concerning this form, notwithstanding the length of time that has elapsed since Lesueur first described it. According to Verrill, Dr. Yarrow collected considerable numbers on the beach at Fort Macon after southwest storms. I was not fortunate enough to obtain any in this way, the single specimen I did secure having been dug out of the sand on Bird Shoal, its habitat being apparently very similar to that of *Aulactinia capitata.*

Fam. CERIANTHIDÆ.

9. Cerianthus americanus, Agass. There can be no doubt that the form found by me belongs to this species, though it differs in some points from the description given by Verrill in his revision from drawings in the possession of Professor Agassiz. Thus, the largest obtained was not as long (20.5 cm.) as Verrill describes (24–28 inches), and the marginal tentacles were only 96 in number, arranged in four cycles, 24 in each cycle. The cinnamon-brown of the column deepened above into purple-brown; the marginal tentacles were of a paler brown, those of the outer cycle being purple; the oral tentacles pure white, unmarked; the funnel-shaped disc bright yellow, with white rays indicating the septa.

Certain of the differences, the number of the tentacles, for instance, may be due to the smaller size of the forms I obtained, and others may be due to Verrill's description having been made not from living specimens, but merely from drawings.

II.

NOTES ON MOLLUSCA OBSERVED AT BEAUFORT, N. C., DURING SUMMERS OF 1882 AND 1884.

By HENRY LESLIE OSBORN, PH. D., Instructor in Zoology at Purdue University.

I have prepared these notes upon the Mollusca of Beaufort, North Carolina, at the request of Dr. W. K. Brooks. In them I have aimed to present a complete account of all the observations of the laboratory party upon the habitat and mode of life of the different forms. These observations are found to be by no means complete. Had the party been especially interested in collecting the Beaufort fauna, the deficiencies in the list would be unpardonable. Such has not, however, been the case; and every one knows how easy it is to overlook all but the most obvious facts when one is not making a detailed study of any problem. We have never dredged thoroughly over the whole harbor and neighboring sounds, and often have not carefully sorted the whole content, the expeditions having been planned to collect some special supply which we knew well where to obtain. The contents of the dredge were well examined at times, and at times not so. The more common and larger forms would be most liable to capture, and smaller and rarer forms would be liable to escape. But, in spite of the various hindrances to completeness, I know that the list comprises the great majority of the forms which can be readily obtained at Beaufort and the place of their occurrence. Many Opisthobranchs were observed; some were sketched and some preserved in alcohol. None of them have been positively identified, and I place them for the present in a supplementary list. I feel reasonably sure that several are new species, but have not at present the material or the literature upon existing forms to justify me in attempting their exact definition. For the identification of the other forms in many instances I am indebted to Prof. Geo. W. Tryon. I have used Dr. Coues' list of the mollusks of the same locality.[1] I take this opportunity also to thank various members of the Chesapeake

[1] Coues. Proc. Philada. Acad. Nat. Sci. 1871.

Zoological Laboratory for various assistance and specimens, and in particular Dr. E. B. Wilson and Dr. J. P. McMurrich for the use of their figures of certain *Nudibranchs* which I have never observed.

LIST OF BEAUFORT MOLLUSKS AS OBSERVED DURING 1882 AND 1884.[1]

	Mud Flats.	Sand Flats.	Bogue Sound.	Wharf Piles.	Salt Marsh.	Outer Beach.	Outside Surface.	Outside Bottom.
CEPHALOPODA.								
Loligo pealii, Les.								r.
PTEROPODA.								
Styliola vitrea, Verrill.							r.	
GASTROPODA PROSOBRANCHIA.								
Eurosalpynx cinerea, Stm.			c.	c.				
Eupleura caudata (Say), H. & A. Ad.			r.					
Purpura haemastoma, Linn., var.			?					
Purpura floridana, Conr.			r.		r.			r.
Latirus sp.						d. r.		
Fasciolaria tulipa, Linn., var. distans Lam.	c.							
Fulgur carica, Conr.		c.	c.					
Sycotypus canaliculatus, Gill.		c.	c.					
Nassa vibex, Say.	c.	c.	c.	c.				
Ilyanassa obsoleta, Stm.	c.	c.	c.	c.				
Olivella mutica, Say.			r.					
Oliva literata, Say.		c.						c.
Anachis avara, Perk.	c.							
Terebra dislocata, Say.		c.						
Bela plicata, Adams.	c.							
Strombus alatus, Gmel.								c.
Ovulum uniplicatum, Sowerb.			c.					c.
Dolium galea, Linn.						d. r.		
Neverita duplicata, Stm.		c.	c.					
Segaretus perspectivus, Say.		c.						
Crepidula fornicata, Lam.	c.	c.	c.	c.				
Crepidula plana, Say.	c.	c.	c.	c.				
Crepidula convexa, Say.	c.	c.	c.	c.				
Vermetus radicula, Stm.		d. c.						
Littorina irrorata, Gray.					c.			
Cerithiopsis terebralis, Adams.	c.							
Cerithiopsis greenii, Verrill.	c.							
Bittium nigrum, Stm.	c.							
Trochus sp.			r.					
Fissurella alternata, Say.			c.					
Chaetopleura apiculata, Carp.			c.					

[1] *C* = common; *r* = rare; *d* = dead shells alone found: in all other cases the living forms were found; *?* = uncertainty as to habitat.

List of Beaufort Mollusks as observed during 1882 *and* 1884.—*Continued.*

	Mud Flats.	Sand Flats.	Bogue Sound.	Wharf Piles.	Salt Marsh.	Outer Beach.	Outside Surface.	Outside Bottom.
LAMELLIBRANCHIA.								
Rocellaria Stimpsoni, Tryon			c.					
Xylotria fimbriata, Jeffreys				c.				
Pholas costata, Linn						d. c.		
Martesia cuneiformis, Gray			c.	c.				
Ensatella americana, Verrill		r.						
Tagellus gibbus, Gray	c.							
Mactra solidissima, Chemn		c.						
Raeta canaliculata, Say						d. c.		
Asaphis coccinea, Mart. juv. ?	r.							
Tellina iris, Say		c.						
Tellina alternata	c.							
Donax fossor, Say						c.		
Venus mercenaria, Linn	c.		c.					
Chione cingenda, Dillw								
Cytherea gigantea, Chemn		c.						
Dosinia discus, Reeve	c.					d. c.		
Cardium magnum, Born		c.	c.			d. c.		
Chama macrophylla, Chemn						d. r.		
Cyclas dentata, Verrill		d. c.				d. c.		
Lucina (Loripes) edentula, Linn								
Solenomya velum, Say	c.							
Scapharca transversa, Verrill	c.		c.					
Modiola plicatula, Lam	c.		c.	c.				
Lithodomus lithophagus, Linn								
Pinna, sp	c.							
Pecten dislocatus, Say	c.		c.					
Anomia glabra, Verrill	c.	c.	c.	c.				
Ostrea virginiana, Lister	c.		c.					

C = common; *r* = rare; *d* = dead shells alone found: in all other cases the living forms were found; *?* = uncertainty as to habitat.

CEPHALOPODA.

LOLIGO PEALII, Lesueur. Trawled several bunches of gelatinous egg-capsules on bottom 5 miles off Fort Macon July 4, 1884. The adults are not common at Beaufort; occasional specimens are obtained.

PTEROPODA.

STYLIOLA VITREA, Verrill. Occasional and in great numbers when they are drifted in from outside. I took a vast number of specimens late in September in '82, at or near midday, on the "young flood" tide. They were taken in surface towing 2 miles off Fort Macon. Mr. Andrews observed them also in '85. This

is the only pteropod which has ever been observed by our party, so far as known. It has been reported as found at Beaufort by our party before my observation of it in '82.

GASTROPODA PROSOBRANCHIA.

EUROSALPYNX CINEREA, Stm. Very abundant, frequenting rock breakwater at Fort Macon; also among barnacles, etc., upon wharf piles. Not noticeably abundant upon the oyster-beds. The eggs are laid 6–15 in number, in cases yellowish in color, leathery in texture, with a short, narrow stalk, and an oval body, containing the eggs. The base is expanded, forming a sort of film which spreads out over a stone or a dead shell, fusing with a similar base from other capsules. There are from five to twenty of these capsules in one brood. They are abundant in early summer, and scarcer during August and later.

LATIRUS *sp.* Large, broken shells, doubtless of this genus, are thrown up on the beach at Cape Lookout, which is more exposed than at Fort Macon.

FASCIOLARIA TULIPA, Linn., var. distans Lam. Very abundant on mud-flats exposed at low tide and over muddy bottoms just beyond low-water mark. Eggs are laid all summer, and are very abundant during June and July. The eggs are deposited in capsules, a variable number in each capsule, and 3–10 capsules in a group. The capsules are almost sessile upon an expanded film of leathery substance spread over a stone, shell, or other convenient anchor. Each capsule is a triangular pyramid in outline, 1 inch high and $\frac{3}{4}$ inch broad at the base, attached by the apex, and with its base somewhat oblong, $\frac{3}{16}$ inch broad and margined with a thin rim of the cartilaginous substance which composes the capsule. Within are often found as many as twenty embryos, held in a thin, transparent, mucilaginous mass. The different embryos in a single capsule are often very unequally advanced in development.

FULGUR CARICA, Conr. Locally known as the *conch*. Specimens of all sizes and ages are extremely abundant everywhere about the harbor. The shape of the egg-cases and capsule-strings is well known. They are found frequently, best upon the outer edges of the sand-flats. I found on June 16 a string most of

the capsules of which contained eggs which had just begun their development. The string was complete and very fresh and clean, and measured 4 feet in length. The largest capsules were 1½ inches in diameter and $\frac{8}{10}$ in greatest thickness. The string was attached by one end to some stone lying in the sand, and floated free beyond this. I do not know which end is formed first. Dr. Brooks informs me that he has good reason to believe that the attached end is oldest and not youngest, as would be the case if Dr. Coues' statement is correct.[1]

Sycotypus canaliculatus, Gill. Much less abundant than *Fulgur carica*, but not rare; found in similar situations with it. I have never met its egg-capsules at Beaufort.

Nassa vibex, Say. Very numerous on the grassy sand-flats at the edges of the salt marshes, where it replaces *Ilyanassa obsoleta;* found also, but less frequently, upon exposed mud-flats.

Ilyanassa obsoleta, Stm. Very abundant upon exposed mud-flats. Found also, but less abundantly, upon sand-flats. Dredged occasionally in sounds, and found among the species about wharves. It is very generally distributed, and occurs in countless numbers. On the sand-bars its shells are very frequently covered, except on the portion that touches the ground, with a species of *Hydractinia.* This is not found on *Ilyanassa* from other situations. On exposed mud-flats at low tides they are present in myriads. The dead shells are used extensively by pagurids.

Olivella mutica, Say. Dredged occasionally in Bogue Sound, but I have never found it in large numbers. It is described in Coues' list as "very abundant," and he gives them the common name of "bead-shells." I have never seen more than ten living specimens.

Oliva literata, Lam. Dead shells are found along outer beaches, and living forms have been dredged both outside and upon the outer edges of sand-flats. Dead and greatly worn shells are very abundant on the beach. This species is not mentioned by Dr. Coues.

[1] Verrill. Rept. U. S. F. C. I, p. 355. (I have frequently captured female specimens with half-laid capsules. The large end is always in the oviduct, while the small end is fastened to some solid body under the sand. The imperfect capsules at the small end act as anchors.—W. K. B.)

TEREBRA DISLOCATA, Say. Living specimens are found in very large numbers upon the outer edges of sand-shoals and in the places where *Renilla* abounds. It is also found, but less abundantly, upon the shallower parts of the shoal, creeping upon the surface, and the dead shells are very common. I have kept them in aquaria and searched among them under their natural conditions, but found no trace of their eggs. It is ACUS DISLOCATUS of Say, as mentioned in Coues' list.

STROMBUS ALATUS, Gmel. I have taken this several times with the trawl in 10 fathoms off Fort Macon, and it appears to be a tolerably common denizen of the bottom several miles off shore. I have never seen *Strombus giganteus* alive at Beaufort, but have found the dead and worn shells cast ashore at Cape Lookout.

OVULUM UNIPLICATUM, Sowerb. There are two varieties of *Ovulum un.* at Beaufort, precisely alike in shape, but markedly unlike in color. One, the yellow-colored variety, lives upon a yellow-colored *Leptogorgia virgulata* found only in the shallow sounds about Beaufort. The other was found upon a carmine-red *Leptogorgia* taken in trawl in 10 fathoms off Fort Macon. Its color is carmine, like that of its *Leptogorgia.* So far as I am aware, it has been noticed but once in Beaufort, but in considerable numbers.[1] The red Pennatulid has never been dredged in the shallow sounds, and the red *Ovulum* has never been seen apart from the red *Leptogorgia*, so far as I am informed.

DOLIUM GALEA, Linn. I have not found the living *Dolium galea*, but have numerous shells found on outer beaches, both at Fort Macon and Beaufort and at Cape Lookout.

NATICA DUPLICATA, Say. Found of all sizes, in great numbers, creeping over the exposed sand-shoals, and often completely exposed at low tide. I have found the egg-masses during June and July. Their shape has been described by Prof. Verrill[2] in his Report on the Invertebrates of Vineyard Sound. They are common upon the shoals in shallow places.

SEGARETUS PERSPECTIVUS, Say. Less numerous than the preceding, but abundant and found in similar situations. I have never found any eggs which I could identify as those of *Segaretus*,

[1] Science, Vol. VI, p. 9, 1885.
[2] Verrill. Rept. U. S. F. C. I, p. 354.

and the specimens I have found on the shoals are always of about the same size—viz., height .57 inch, width 1.5 inches.

CREPIDULA FORNICATA, Linn. Very common. Found very generally on old specimens of *Limulus*, and found attached to shells and stones almost anywhere where these may be found. I have noticed this species on *Fulgur*, *Sycotypus* and *Ostrea*. They grow very large on *Limulus*, and measure sometimes two inches in their greatest dimension. In this and the two following the eggs are laid in a gelatinous mass, which is composed of a number of small, finger-shaped bodies held together at one common point much after the fashion of a cluster of *Loligo* eggs. These egg-masses were never found independent of the animal, but always under the parent, between it and the surface adhered to. They are very commonly found in this situation in the summer months. Removal of the eggs from the care of the parent offers no obstacle to their development.

CREPIDULA PLANA, Say. Less abundant than *C. fornicata*, but not rare, and found in similar situations. It is very often found adhering to the inner surfaces of dead shells, and greatly misshapen in consequence of adaptation. In increased growth they tend to assume greatly elongate forms, while *C. fornicata* becomes very broad as it grows larger. Its eggs are frequently met; they are in capsules like those of *C. fornicata*, and are protected by the parent in a similar manner.

CREPIDULA CONVEXA, Say. Found frequently with the preceding, and its eggs are met in similar circumstances.

LITTORINA IRRORATA, Say. Very abundant at Beaufort. It frequents the marshes, creeping over the stems of the marsh-grass, and often may be seen on the leaves above the water-level.

L. littorea is very common at Hampton; may be found in unlimited numbers in Hampton Harbor. I do not know that it has hitherto been recorded so far south. Verrill has recorded it from Southern New England.[1] I do not recollect to have ever seen it at Beaufort.

CERITHIOPSIS TEREBRALIS, Adams. Found in large numbers upon stems of eel-grass growing in the border between the mud-flats and the channels in company with the following.

[1] Verrill. Proc. U. S. Nat. Mus. III, p. 409, 1881.

CERITHIOPSIS GREENII, Verrill. Very numerous in situation with foregoing.

TROCHUS (?), *sp.* Single specimen undetermined, but apparently a species of *Trochus* dredged in Bogue Sound.

FISSURELLA ALTERNATA, Say. Dredged frequently in Bogue Sound; few taken in almost every haul. Found best on bottom with dead shells of *Venus* and *Ostrea*, and usually found upon these. The animals live well in an aquarium; but, though I have captured them at all times from June 1st till October, I have never seen their eggs or been able to obtain any from animals kept in confinement.

CHAETOPLEURA APICULATA, Carp. Taken in Bogue Sound very commonly, but never many at a time. They are found of all sizes—from very small ones: length .3 inch to 1 inch—but the eggs have not been discovered at Beaufort nor the animal been observed to breed in confinement.

LAMELLIBRANCHIA.

ROCELLARIA STIMPSONI, Tryon. We have dredged in Bogue Sound on barren bottoms, furnishing for the most part deserted shells of *Ostrea* and *Venus*—huge shells of *Venus mercenaria*, which were riddled with holes, and in which could be found the burrows of *Rocellaria* lined with cemented bits of broken shells.

XYLOTRIA FIMBRIATA, Jeffreys. Very common and very destructive. I have a large section from a pile which had been in use but two years, and yet is completely honeycombed by these destructive creatures. The same pile was bored into by the following species. The *Xylotria* does not come to the surface; its burrows run in a sinuous course longitudinally of the log, and they do not cross each other's path, though they wind around each other so as to produce a complete network of tubes. These tubes are often quite long, and they extend finally outward to the surface, where they taper and open by a very small hole, out of which the palets may often be seen to protrude slightly. The burrow is made by the shell, which is at the end opposite the palets, and is lined with calcareous material.

PHOLAS COSTATA, Linn. I have never found this alive at Beaufort, but I have found numerous shells and fragments of

shells thrown up on the outside beach at Fort Macon. According to Dr. Gould, this species has been found buried in 2–3 feet of mud in New Bedford (Mass.) Harbor. The fact that the shells are found nowhere at Beaufort except on the outer beach, leads one to suspect that its situation is not in the mud-flats at Beaufort.

MARTESIA CUNEIFORMIS, Say. Found in Bogue Sound boring in dead shells of *Venus*. Very abundant.

ENSATELLA AMERICANA, Verrill. Found rarely buried one foot or thereabout beneath the surface in the clean sand or sand-shoals. Dead shells are very numerous on the shores of the shoal. My specimens are only about three inches in length.

TAGELLUS GIBBUS, Gray. Shells found in enormous numbers, and the animals very common; found buried about a foot below the surface in mud-flats between tides.

MACTRA SOLIDISSIMA, Chemn. Found by digging on the outer edge of sand-shoals; probably common there, for the shells are very common on the edges of the beaches of such shoals.

RAETA CANALICULATA, Say. I have never seen this species alive at Beaufort, but the delicate shells, always broken, are thrown ashore in great numbers on the outer beach. It is probably abundant in the deep water outside.

ASAPHIS COCCINEA, Mart. juv.?

TELLINA IRIS, Say. I have an unlabeled specimen from Beaufort, but cannot recall its situation.

TELLINA ALTERNATA, Say. Not found in the mud-flats near the laboratory, but very abundant in similar mud-flats along the channel, and farther towards the outside. It is found on this outer flat with *Dosinia discus*, which also never occurs in the mud near the laboratory. It is obtained by digging, and lives upon flats exposed at low water.

DONAX FOSSOR, Say. Very abundant. It lives in the pure sand upon the outer beach, about six or eight inches below the surface, and in the zone which is uncovered at low tide.

VENUS MERCENARIA, Linn. Dug in large numbers in the flats, and buried in the edges of sand-shoal to await sale.

CHIONE CINGENDA, Dillw.

CYTHEREA GIGANTEA, Chemn. Found abundantly and of all sizes—from one inch long to six inches—upon the surface of the

sand-flats in the outer edges; never met in the mud-flats, and never, so far as I recall, dredged or taken in the trawl in the sounds, or outside or about the mud-flats.

DOSINIA DISCUS, Reeve. Found sparingly in situations with *Tellina alternata*; dead shells found very commonly on outer beach and beach of sand-shoal.

CARDIUM MAGNUM, Born. Dead shells are extremely abundant upon the fort beach and the beaches bordering the sand-shoals, and the animals are taken in the dredge in Bogue Sound, and sometimes found on Bird Shoal, on its outer edges.

CYCLAS DENTATA, Verrill.

SOLENOMYA VELUM, Say. Extremely abundant in the mud-flats near laboratory between tides.

SCAPHARCA TRANSVERSA, Verrill. Found in the shallow water on the edge of the mud-flats, among the eel-grass; also dredged in Bogue Sound. Never very abundant, but by no means rare.

LITHODOMUS LITHOPHAGUS, Linn. Numerous specimens are found boring into the surface of wharf-piles beneath the bark. They do not appear to do any sensible damage. The same piles are thoroughly honeycombed by *Xylotria*.

PINNA SEMI-NUDA. This species is abundant in the flats which are near the laboratory. They do not grow to great size, the largest ones being not over seven inches long by four or five broad.

PECTEN DISLOCATUS, Say. Found in the eel-grass bordering the channel; in the mud-flats near the laboratory; also dredged in Bogue Sound.

ANOMIA GLABRA, Linn. Very common; attached to dead shells of *Ostrea*, *Venus*, and other Mollusca. Taken on the mud-flats; also on Bogue Sound and the sand-flats. The dead shells are washed upon the beaches in considerable numbers.

OSTREA VIRGINIANA, Lister. Very common at Beaufort. On the great beds east of the town the long and narrow oysters stand with their axis vertical, and grow as closely packed as they can stand. The number, if stated, would be too large to convey any meaning; and the water is teeming with the spawn. A stone placed in a wire cage and sunk on the flats was completely paved with spat in three weeks, and in two months these had attained a size of .83 inch x .52 inch in the case of very many individuals.

The following species are not mentioned in Coues' list:
Loligo pealii, Lesueur.
Styliola vitrea, Verrill.
Oliva literata, Say.
Strombus alatus, Gmel.
Ovulum uniplicatum, Sowerb.
Rocellaria stimpsoni. (Prob. = Coues' *Gastrochæna sp.*)
Xylotria fimbriata, Jeffreys.
Martesia cuneiformis, Gray.
Donax fossor, Say. (Prob. = *D. variabilis* of Coues' list.)
Chione cingenda, Dillw.
Lithodomus lithophagus.

I present with this *résumé* of our information of the Beaufort mollusks a list of all the forms of Opisthobranchs which we know to occur at Beaufort, with as full description as the preserved material and notes and drawings of the different members of the party will permit.

Aplysia, *sp. indet.* Dated July 4, 1882. My note upon the colored sketch reads: "Length 11 inches; color outside, light olive-brown with large spots of flesh-color; covered with warty-looking white patches; purplish hue showing through transparent skin. Color within epipodia less interrupted by white patches. Mantle decidedly purple. Gills flesh-color with black." Specimens are brought in not infrequently by fishermen, who capture them outside. They are known as the "sea-mule." I have seen perhaps half a dozen like the one described above, and one specimen probably *Aplysia*, but quite different from the above. It is 2½ inches long; body in front of epipodia much shorter; tentacle not so elongate; the surface has a very wrinkled texture, with dark lines following the wrinkles; general color olive-brown interrupted with patches of white. It is very possibly a young specimen of the larger form.

Doris, No. 1. Single specimen found and figured June 24, 1882. Body oval, flat below and arched dorsally; foot entirely covered by the mantle-edge, also head; single pair of tentacles, laminate, retractile, black below, the laminæ white; mantle tinged brown, yellowish on the margin; uninterrupted yellow band; surface closely covered with tubercles, large, low, blackish in color, giving very warty appearance; gill composed of seven

main stalks, each bearing six to eight branchlets; color brownish, very deep along main stalks; gill very contractile and retractile. Length of body measured over mantle to base of gills $2\frac{8}{10}$ inches; width $1\frac{4}{10}$ inches. Gills expand to length of $1\frac{2}{10}$ inches. I have no record as to the locality of the specimen; think it was dredged in Bogue Sound.

DORIS, No. 2. Several forms were obtained in the same locality and on dead shells, and are probably identical with four found by Dr. Osborn. From a drawing the following points may be added: Color is yellowish, with brownish dots irregularly scattered over surface of the mantle, more especially towards the periphery. Spicules confined mainly to the central area of mantle. Oral tentacles and gills orange-yellow; eyes present.—*J. P. McM.*

Another *Doris*, not included in Dr. Osborn's list, was obtained in the summer of 1885. One or two specimens were brought up by the trawl in Bogue Sound. Oral tentacles represented by a veil, dorsal tentacles not perfectly retractile, branchiæ not retractile. Mantle voluminous, completely covering the foot, and possessing numerous tubercles comparatively high and varying somewhat in shape. Color of entire mantle was pale bluish-green or gray. Length of alcoholic specimens, about $\frac{3}{4}$ inch; breadth $\frac{1}{2}$ inch.—*J. P. McM.*

ÆOLID, No. 9. Length $\frac{1}{2}$ inch.—*J. P. McM.*

ÆOLID, No. 10. Length about 5 mm. Common or *Sertularia*.

DORIS, No. 2. Single specimen found adhering to dead clam-shell dredged in Bogue Sound July, 1884. Mantle voluminous, tuberculated, the tubercles low, blunt, not so close as in No. 1; surface covered with stony spicules. Gills and dorsal tentacles wholly retractile. Color brownish. No sketches preserved. Specimen (alcoholic) $1\frac{25}{100}$ inches long, $\frac{68}{100}$ inch wide.

DORIS, No. 3. Single specimen, labeled "Beaufort, July 26, 1882." It is brown, tuberculated, the head marked off from the foot very completely and with the mouth shutting in above both. Gills and tentacles wholly retractile; the former of five branches bearing "pinnate" branchlets. Length $\frac{64}{100}$ inch. Not very distinct from No. 2, but very considerably smaller; the tubercles very much finer.

OLIVA LITERATA. Somewhat abundant on the outer shoals, where living specimens were obtained.—*J. P. McM.*

FISSURELLA ALTERNATA. Very common in the "slues" off the eastern end of Bird Shoal, on the oyster-rocks, associated with sponges.—*J. P. McM.*

ANCULA, *sp. nov.*, Verrill. Three or four specimens of this form have been found at Beaufort. Dr. E. B. Wilson found and figured one in 1880. The notes upon the specimen read as follows: "Steamboat Wharf, May 18, 1880. Three tentacles on each side in front, and only three lateral tentacles. Body very changeable in shape. Colors very vivid on hydroids. Very active. About twenty rings on tentacle; gills contractile, not wholly retractile; no eyes; surface papillose, papillæ light-colored; gills lighter at edges, darker centre." Wilson's sketch, a copy of which is in my possession, shows the body dark, with the rhinophores dark, and the two other head-tentacles and the lateral tentacles bright blue, interrupted by a zone of bright yellow in the middle. This form was pronounced by Prof. Verrill, from Wilson's sketch, to be a new species of *Ancula*. I have never seen it at Beaufort at all abundant, but found a specimen rarely, and sketch agrees with Wilson's description, with the addition that the posterior end of the foot is blue, the color of the tentacle, and the very tip is bright yellow. There are five main gill-lobes, subdivided into numerous branches. My figure shows three lateral tentacles on the left and four on the right, and Wilson's sketches of dorsal and ventral view show four lateral tentacles upon the left side. I did not observe any styliform appendages upon the rhinophores. Length .9 inch, and breadth just in front of gills .2 inch.

ÆOLIDÆ. I have figures and notes, or preserved specimens, of ten species of Æolids from Beaufort. Some are well enough defined for recognition; but on all of them my information is at present most fragmentary. I will describe them as far as possible, hoping in the future to be able to complete this detached account.

No. 1. Very common among the *Sertularia pumila* thrown up after storms. It is apparently a species of the genus *Æolis*. Foot long and narrow, and *tentacularly* produced anteriorly on each side. Tentacles, two pairs; one pair strongly ringed and not retractile. No evidence of eyes in alcoholic specimen. Gills in several—about four—groups very readily falling off. Jaw com-

posed of delicate plate upon each side; margin completely toothed; teeth blunt and very even. Odontophore composed of single row of teeth, about fifteen in row. Each tooth shaped like upper beak of parrot; four small denticles; on each side a strong median one. No record of color. The specimens average .3 inch in length, with occasional specimens larger, and many smaller.

No. 2. Found in large numbers upon a floating log in July, 1884. I have only alcoholic specimens to describe from. These were in life .4–.6 inch long. The foot is long and not very narrow; there is no evidence of tentacular prolongation of sides of it in front. The tentacles neither give evidence of being ringed; they are not retractile. The gills are in a continuous row down both sides of the back, and are very numerous; well separated along the dorsal middle line. No eye shows in the alcoholic specimens. The color was brown, faintly reddish, due to the gills, the body itself being hardly colored. The jaw is made up of two very strong pieces, one on each side, supported by a continuous row of short, blunt teeth upon the biting margin. The odontophore is made up of a long row of teeth in a single series; each tooth has one strong central tooth, and about five finer, sharp-pointed teeth on the sides of the central tooth, all united in one single plate shaped much as in No. 1.

No. 3. No specimens, but colored sketches of a species frequent upon the bark of wharf-piles, and upon bryozoa and hydroids attached to these. I have figures of what would appear to be three species. One of these has the foot angularly produced anteriorly, and two pairs of tentacles, neither ringed; has a black eye-spot just behind the hinder pair of tentacles, and a small patch of bright vermilion-red just under the skin on each side of the head between the tentacles. Tentacles very faintly yellowish-tinged. The general color is brown-gray. The gills are in nine transverse rows, three to four on each side, and each gill is dark brown (Vandyke brown), internally tipped with lighter brown, and interrupted almost at the tip by a small patch of brilliant vermilion-red. Length .57 inch.

No. 4. Figure from a specimen found upon *Eudendrium* at steamboat wharf in Morehead City, July 12, 1884. This was much more highly colored than the specimens of No. 3 I have

seen, as well as unlike them in shape, and is probably generically distinct. The general color of the body was light brown; of the foot, flake-white; of the posterior end of the body, bright blue. The form was elongated; the foot angularly produced in front; the tentacles two, not ringed, deeper brownish in color. At the extreme front a bright, narrow band of vermilion started and ran backward down the middle line about one-tenth the length of the body. The gills were very numerous, and bunched in three sets upon each side. The filaments were very long and narrow, and finely tapering. The filaments were all of them vividly colored: very dark brown below, surmounted near the top by a narrow band of bright vermilion, this by an equal band of bright yellow, and finally the tentacle tipped with flake-white. I have no note upon the size of this specimen, but, as I recall it, it was not over $\frac{3}{10}$ inch in length.

No. 5. One specimen figured was taken in Bogue Sound June 17, 1884. It is much like No. 3, except in respect to the tentacle. Body elongate, tapering behind; color brownish, very light and transparent. Foot angularly produced anteriorly. Gills about the same as in No. 3 as to disposition, also as to color. The posterior pair of tentacles are foliated, with a narrow stalk and club-shaped outer portion; the central rachis is pure flake-white, as also the tip; the body of the tentacle is brilliant vermilion; the color of body of the tentacle is interrupted by rings of the same white as the stem and tip. Length $\frac{67}{100}$ inch.

No. 6. FACELINA PILATA, Verrill and Emerton. Dated May 18, 1880. Specimen found and figured by Dr. Wilson, and named by Prof. Verrill. In the figure, five bright red spots surrounded by white ring, upon brownish ground, show along the dorsal regions: one behind, the other well forward; a second two farther back, and a single one about half-way of the body's length. Wilson's note says: "Areas about spots pearly white. Tentacles scarcely ringed, though there are obscure indications of them. Dorsal gills obscurely arranged in five rows, but posteriorly the rows are very indistinct. Body with a prominent swelling, which pulsates with heart beneath." Length .4 inch. I have never met this species at Beaufort.

No. 7. FACELINA, *sp. nov.*, Verrill. A specimen labeled "*Vacillaria, sp. nov.* (Verrill)," is figured by Wilson. The name

Vacillaria has no doubt been accidentally substituted for *Facelina*. The view is a ventral one, but the character figured indicates without doubt *Facelina* as the genus. The anterior end of the foot drawn out into very finely tapering tentacular processes; a pair of head-tentacles, also a pair of dorsal ringed tentacles. The rings strong, and eleven in number. Gills are present upon the dorsal surface; these seem to be disposed in five sets. They are tipped with blue, and have a narrow ring of red just below the tip. The remainder of the gill and the general surface are grayish. Posteriorly the foot is tipped for one-third its length bluish. Length 1.1 inches; rhinophore .2 inch long.

No. 8. FACELINA PILATA, Verrill and Emerton. One of three Æolids figured by Dr. J. P. McMurrich, in July, 1885, which I will describe from his figures. It is in shape and color very nearly like No. 7, and probably is identical with it. It is a dorsal view. Foot angular in front; anterior tentacles yellowish-tipped; dorsal tentacles ringed. Eyes present behind dorsal tentacles. Body tinged bluish (Wilson's is figured as bluish posteriorly). Gills in four clusters, reddish tipped with blue, brighter red just below the tip. Length .4 mm.

No. 9. Dated Beaufort, N. C., July, 1885. Body tapering. Foot tentacular anteriorly. Two pairs tentacles; dorsal ones not ringed, simple. Eyes present behind dorsal tentacles. Strip of red on each side of head, and on body behind dorsal tentacles. Gills very numerous, in ten fairly distinct rows; four or five short filaments in each row, on each side. Filaments all colored alike: blue-purple below, tipped with yellow. Foot bluish, with decided blue posteriorly. Faint reddish line down middle of dorsal region behind gills. Size not given. This species agrees fairly well with *Montagua vermifera*, Verrill,[1] excepting in the colors, which are less vivid than here, but with much the same distribution.

No. 10. Specimen, figured by McMurrich and labeled "common," is one I have never seen. The foot is broadly elongate, slightly angled in front, bluish. Bluish patch upon front of head in middle line. Anterior tentacles very long, not sharp-pointed; posterior, simple shorter. Body 4-lobed, each lobe bearing about six slender, obtusish gills; each lobe with a ring

[1] See Vineyard Sound Report, I, p. 666.

of darker brownish color. The gill-filaments brownish-colored, interrupted by short, lighter patches.

Fam. TRITONIADÆ.

Scyllaea, *sp. indet.*[1] One specimen found thrown upon Fort Macon beach after an easterly gale July 5th, 1884. Foot long and very narrow and grooved. Body strongly compressed laterally and terminated posteriorly by vertical, fin-like, flattened portion; two lateral expansions on each side, thin, and with the edges very delicate, very mobile. Single pair of tentacles, retractile into sheaths broadly expanded above, with the posterior edge very delicate and wavy. Gills very gauzy, white, spread out on short stalk, numerous—eight or nine on the dorsal surface of each lateral mantle-lobe. Body light brown, becoming very light—almost pure white—on the delicate edges of the hinder part of tentacle-shields, lateral expansions and tail-fin. Natural habit is creeping upon the stems of *Laminaria;* but the creature swims readily, with fish-like motion. Measurements (from drawing): Length 2 inches; height at middle .4 inch; epipodia, length from body to tip .7 inch; width, .2 inch; tentacle-shields, height .4 inch; much expanded at tip: width .24 inch; tail-fin, height .5 inch.

Fam. PHYLLIDEIDÆ.

Pleuro-phyllidia *sp. indet.* Extremely abundant upon the outer edge of Bird Shoal in association with *Renilla.* Dredging for the latter always brings a number of specimens of this; and they are abundant during the entire summer. This description is from my notes on living specimen, made June 5, 1882: "Color gray; foot lighter than mantle; both margined with white, and the mantle marked with a central and eight or ten lateral longitudinal broad white bands which run its entire length, and seven narrow bands of white lying in the spaces between the broad ones and not extending the whole length of the mantle. Gills very short, in many rows on the under side of the mantle. Two short, knobbed tentacles. Length $1\frac{5}{10}$ inches; breadth $\frac{5}{8}$ inch." The tentacles are situated close together in the middle line in

[1] See Science, Vol. VI, p. 9, 1885.

front and are tipped with very numerous white, fungus-like plaits. The gills are brown, as well as the body in the mantle-groove, and the exposed parts of the foot and head are dark gray, the color of the body above. The dentition is 50–150. The median tooth is broad, with a single strong median denticle and ten smaller denticles on each side, and with about two feebly developed denticles on the slope of the large median one. The lateral rows are composed of claw-shaped simple teeth, and there are very many in each row—in the fullest row as high as fifty. The jaws are strong and cavernous, the margin simple.

III.

NOTES ON ECHINODERMS OBTAINED AT BEAUFORT, N. C. By H. F. NACHTRIEB.

STARFISH: *Luidia clathrata and Astropecten articulatus.* Are both found out in the ocean, especially off Shackleford Beach. They probably spawn early in the spring—before June. The larvæ I raised were raised from eggs artificially fertilized during August (Aug. 14th). The reproductive organs of the starfish at this time contained *very* few mature reproductive elements. These organs did not increase in size during the summer. *Asterias Forbesii* (?) is found about the town-wharf in spring, and during the summer up Bogue Sound near Carolina City, in the bay at Cape Lookout, and various other places. This species is particularly migratory, and was not sufficiently studied to make out its habits. It probably spawns some time before May, or early in May.

OPHIURIDS: *Ophiophragmus Wurdemanii.* Habitat: Sandy mud along low-water mark off Shark Shoal, opposite freight depot. Stray specimens were found in other parts of Shark Shoal, and a very few specimens were found on Bird Island Shoal. Spawning-time seems to be about September. My material for the development was obtained from eggs artificially fertilized in the first week of September (Sept. 7th). Develops into a young ophiurid within forty-eight hours. Development without pluteus.

Ophiothrix angulata. Habitat: Cavities of sponges; especially abundant in sponges found near "Green Rock." Seems to spawn during the summer. Mature reproductive elements can be obtained during July, August and September.

Ophiura brevispina, var. *Olivacea.* Habitat: Among the eel-grass on the muddy bottom in front of the Gibbs House, and in all other parts of the harbor with a similar bottom. The most abundant and most easily obtained species. Seems to spawn during the summer. My material was obtained during July (July 7th–9th).

Another undetermined species was found out on the ocean-bottom. I think there are several specimens of this in the laboratory. It is one with a well-developed and beautifully symmetrically arranged system of plates.

ECHINOIDS: *Mellita testudinata.* Bird Island and Shark Shoals and other sandy bottoms; both in places uncovered at low tide and in places always under water. Very abundant at a depth from a foot to several fathoms. Spawns early in summer —June and July; perhaps also before this.

Moira atropos. Abundant on Shark Shoal opposite freight depot of Morehead City. Also found on other neighboring shoals. None or but very few on Bird Island Shoal. Spawns in summer. My material is from eggs spawned August 22d.

Arbacia punctata and *Strongylocentrotus droebachiensis* are found up Bogue Sound near Carolina City, and in various other parts of the harbor, etc. Both spawn early in summer.

HOLOTHURIANS: *Cucumaria* ———*?* Mud in front of laboratory and on Shark Shoal. Spawns during the summer.

Thyone ———*?* In company with *Cucumaria*, but much less abundant. Probably also spawns during the summer. Both species throw off only a few ova at a time.

Synapta ———*?* Sandy mud of various shoals about Shark Shoal. Not very abundant. Either spawns during the winter or early in the spring—very likely early in the spring.

IV.

A LIST OF THE FISHES OF BEAUFORT HARBOR, N. C.

By O. P. JENKINS, De Pauw University, Ind.

In the summer of 1886, while attending the session of the Marine Laboratory of the Johns Hopkins University, held at Beaufort, N. C., I made a collection of fishes of the harbor.

My collection contained about eighty species, which, together with some others which, on account of their size or other reason, I was unable to preserve, made in all eighty-five species which came under my observation.

In the Proceedings of the Philadelphia Academy of Natural Sciences for 1877, Dr. Yarrow published a list of the fishes observed by him and Dr. Coues in Beaufort Harbor and vicinity.

In the Proceedings of the U. S. National Museum for 1879, Dr. Jordan and Prof. Gilbert published a list of the fishes of Beaufort Harbor, which contained both those of Dr. Yarrow's list and those which they added from a collection made by them in the summer of 1878.

In the following list are given those of the above lists, with the addition of twenty-three species which are in my collection, but do not occur in either of the lists mentioned.

A separate list of these species is appended to the general list.

In the following list there are omitted certain species of the former lists which are now no longer recognized by authors.

The numbers in parentheses following the specific name are those of Dr. Jordan's "Catalogue of the Fishes Known to Inhabit the Waters of North America" (1885, Washington).

The numbers following these, marked S., are those of Jordan and Gilbert's "Synopsis of the Fishes of North America" (1882, Washington).

The fishes of my collection have, for the most part, been deposited in the museum of the Indiana State University.

Dr. Jordan has had the kindness to examine the collection, and to give me aid in studying it.

1. BRANCHIOSTOMIDÆ.

1. *Branchiostoma lanceolatum* (Pallas), (1). Synopsis 1. Lancelet. Found on Bird Shoal. (Extremely abundant in Hampton Roads, Va.—W. K. B.)

2. Galeorhinidæ.

2. *Carcharhinus terræ-novæ* (Richardson), (44). S. 30. Sharp-nosed Shark. Abundant. Obtained one specimen seven feet in length.

3. Sphyrenidæ.

3. *Sphyrna tiburo* (Gill), (45). S. 31. Shovel-headed Shark. Abundant, although less so than the preceding.

4. *Sphyrna zygæna* (Lin.), (47). S. 32. Hammer-headed Shark. One specimen recorded by Dr. Yarrow.

4. Odontaspididæ.

5. *Carcharias littoralis* (Mitchill), (49). S. 34. Sand Shark. One specimen recorded by Jordan and Gilbert.

5. Pristidæ.

6. *Pristis pectinatus* Latham (56). S. 44. Saw Fish. Three specimens were seen. Obtained a "saw" 39 inches in length.

6. Raidæ.

7. *Raia lævis* Mitchill (75). S. 52. Barndoor Skate. Recorded by Dr. Yarrow as common. Not seen by me.

7. Torpedinidæ.

8. *Torpedo occidentalis* Storer (76). S. 46. Torpedo. A fisherman described to me the catching and experimenting with a torpedo.

8. Trygonidæ.

9. *Pteroplatea maclura* (Le Sueur), (83). S. 59. Butterfly Skate. Abundant.

10. *Trygon sayi* (Le Sueur), (87). S. 62. Sting Ray. Abundant.

9. Myliobatidæ.

11. *Stoasodon narinari* (Euphrasen), (92). S. 66. Bishop Ray. One large specimen seen by Jordan and Gilbert.

12. *Miliobatis freminvillei*, Le Sueur (94). S. 67. Eagle Ray. Recorded by Jordan and Gilbert.

10. Cephalopteridæ.

13. *Manta birostris* (Walbaum), (97). S. 70. Devil Fish. Two specimens were caught at Cape Lookout.

11. Acipenseridæ.

14. *Acipenser brevirostrum* Le Sueur (105). S. 78. Sturgeon. Recorded by Jordan and Gilbert.

12. Lepidosteidæ.

15. *Lepidosteus osseus* (Lin.), (107). S. 80. Gar Pike. One specimen caught off the wharf by Dr. Brooks.

13. Siluridæ.

16. *Galeichthys felis* (Lin.),,(138). S. 110, 111. Sea Catfish. Common. A fisherman brought to Dr. Brooks a specimen with several large eggs in its mouth.

17. *Ælurichthys marinus* (Mitch.), (141). S. 112. Gaff-topsail. Recorded by Jordan and Gilbert.

14. Albulidæ.

18. *Albula vulpes* (Lin.), (429). S. 431. Lady Fish. Reported by Dr. Yarrow on the authority of a fisherman.

15. Elopidæ.

19. *Elops saurus* Lin. (433). S. 435. Horse Mackerel. One large specimen seen by Jordan and Gilbert.

20. *Megalops atlanticus* Cuv. and Val. (434). S. 436. Jew Fish. Reported by Dr. Yarrow on the authority of a fisherman.

16. Clupeidæ.

21. *Clupea mediocris* Mitch. (443). S. 443. Hickory Shad. Reported by Dr. Yarrow.

22. *Clupea vernalis* Mitch. (444). S. 444. Alewife. Reported by Dr. Yarrow.

23. *Clupea sapidissima* Wilson (446). S. 446. Common Shad. Reported by Dr. Yarrow as common in the Neuse River.

24. *Opisthonema oglinum* (Le Sueur), (451). S. 448. Thread Herring. Obtained a number of specimens at Cape Lookout.

25. *Brevoortia tyrannus* (Latrobe), (453). S. 450. Fat Back, Menhaden. Very abundant.

17. ENGRAULIDÆ.

26. *Stolephorus browni* (Gmelin), (460). S. 453. Sardine, Anchovy. Abundant.

18. SYNODONTIDÆ.

27. *Synodus fœtens* (Lin.), (477). S. 463. Sand Pike. Very common.

19. CYPRINODONTIDÆ.

28. *Cyprinodon variegatus* Lacépède (545). S. 525. Common.

29. *Fundulus majalis* (Walbaum), (557). S. 532. Rock Fish. Very common.

30. *Fundulus heteroclitus* (Lin.), (566). S. 543. Killifish. Common.

31. *Gambusia patruelis* (Baird & Girard), (585). S. 551, 552, 562. Common.

20. CONGRIDÆ.

32. *Conger conger* (Lin.), (636). S. 588. Conger Eel. Obtained two specimens.

21. ANGUILLIDÆ.

33. *Anguilla anguilla rostrata* Le Sueur (638). S. 587. Common Eel. Common.

22. BELONIDÆ.

34. *Tylosurus hians* (Cuv. & Val.), (654). S. 696. Gar Fish. Common.

35. *Tylosurus marinus* (Bloch and Schneider), (660). S. 599. Not distinguished from the preceding by the fishermen. Common.

36. *Scomberesox saurus* (Walbaum), (663). S. 601. Saury. Recorded by Dr. Yarrow.

37. *Hemirhamphus unifasciatus* Ranzani (665). Obtained numerous specimens at Cape Lookout.

38. *Halocypselus evolans* (Lin.), (672). S. 606, 607. One young specimen obtained by Jordan and Gilbert.

39. *Exocœtus heterurus* Rafinesque (677). S. 610, 613. Flying Fish. Seen by Dr. Yarrow.

23. Syngnathidæ.

40. *Siphostoma floridæ* Jordan and Gilbert (689). S. 615*b*. Pipe Fish. Common.

41. *Hippocampus punctulatus* Guichenot (697). S. 619*b*. Sea Horse. Obtained four specimens. Common in eel-grass. W. K. B.

24. Fistulariidæ.

42. *Fistularia tabaccaria* Lin. (702). S. 622. Trumpet Fish. Seen by Dr. McMurrich in summer of 1884.

25. Mugilidæ.

43. *Mugil cephalus* Lin. (715). S. 633, 634. Jumping Mullet. Very abundant.

44. *Mugil curema* Cuv. & Val. (717). S. 635. White Mullet. Common. Jordan and Gilbert.

45. *Qurerimana gyrans* Jordan and Gilbert (721). A very small fish, thought by the fishermen to be the young of the Mullet or Blue Fish. Obtained numerous specimens from a large school at the wharf at Shackleford Banks.

26. Atherinidæ.

46. *Atherina carolina* Cuv. and Val. (724). S. 636. Friar. Reported by Coues and Yarrow.

47. *Menidia menidia* (Lin.), (734). S. 644. Silver Side.

27. Sphyrænidæ.

48. *Sphyræna borealis* De Kay (739). S. 649. Barracuda. "Young specimens common." Jordan and Gilbert, W. K. B.

28. Echeneididæ.

49. *Echeneis naucrates* Lin. (750). S. 657. Pilot. Three specimens were obtained, two from sharks and one caught by hook and line.

50. *Remora remora* (Lin.), (752). S. 658. Remora. Seen by Dr. Yarrow.

29. ELACATIDÆ.

51. *Elacate canada* (Lin.), (756). S. 661. Cabio. Crab-eater. Occasionally brought in by fishermen. One specimen obtained. W. K. B.

30. XIPHIDÆ.

52. *Xiphias gladius* Lin. (757). S. 662. Sword Fish. Occasionally reported by fishermen. W. K. B.

31. TRICHIURIDÆ.

53. *Trichiurus lepturus* Lin. (760). S. 666. Hair Tail. One specimen brought to the laboratory. W. K. B.

32. SCOMBRIDÆ.

54. *Scomberomorus maculatus* (Mitch)., (767). S. 671. Spanish Mackerel. Common.

55. *Scomberomorus regalis* (Bloch), (768). S. 672. Cero. Reported by Dr. Yarrow.

56. *Sarda sarda* Bloch (771). S. 674. Bonito. Reported by Dr. Yarrow.

57. *Orcynus thynnus* (Lin.), (774). S. 677. Horse Mackerel. Two specimens seen.

33. CARANGIDÆ.

58. *Caranx bartholomæi* Cuv. and Val. (784). S. 687, 688. Obtained several specimens.

59. *Caranx chrysus* (Mitch.), (785). S. 685. Crevallé. Common.

60. *Caranx latus* Agassiz (786). S. 690. Obtained a few specimens.

61. *Caranx hippos* (Lin.), (787). S. 691. Reported by Dr. Yarrow.

62. *Caranx crinitus* Akerly (790). S. 692. Cobbler Fish. Reported by Dr. Yarrow.

63. *Vomer setipinnis* (Mitch.), (791). S. 694. Moon Fish. Obtained a number of specimens.

64. *Silene vomer* (Lin.), (793). S. 693. Moon Fish. Obtained a number of specimens.

65. *Chloroscombrus chrysurus* (Lin.), (794). S. 695. One young specimen obtained.

66. *Trachynotus carolinus* (Lin.), (796). S. 696. Pompano, Sun Fish. Common.

67. *Trachynotus rhomboides* Bloch (800). S. 697. One specimen obtained.

68. *Naucrates ductor* (Lin.), (803). S. 700. Pilot Fish. Given in Dr. Yarrow's list on the authority of a fisherman, but omitted by Jordan and Gilbert as being easily confounded with the next. Not seen by me.

69. *Seriola zonata* (Mitch.), (804). S. 704. Rudder Fish. One specimen obtained by Dr. Yarrow.

34. Pomatomidæ.

70. *Pomatomus saltatrix* (Lin.), (814). S. 708. Blue Fish. Abundant.

35. Nomeidæ.

71. *Nomeus gronovii* (Gmelin), (815). S. 709. One young specimen about one-fourth of an inch in length caught with the tow-net, swimming free. In the fall it is common; then found only in a medusa (*Stomolophus*), the medusa never being found without a *Nomeus*.

36. Stromateidæ.

72. *Stromateus triacanthus* Peck (819). S. 712. Dollar Fish. Reported by Coues and Yarrow.

37. Serranidæ.

73. *Roccus septentrionalis* (Bloch and Schneider), (954). S. 830. Striped Bass. Reported by Dr. Yarrow.

74. *Roccus americanus* (Gmelin), (957). S. 833. White Perch. Reported by Dr. Yarrow as abundant in the New and Neuse Rivers.

75. *Serranus atrarius* (Lin.), (958). S. 836. Black Fish. Abundant.

76. *Serranus philadelphicus* (Lin.), (960). S. 837. Obtained a few specimens.

77. *Serranus subligarius* (Cope), (963). S. 839. Obtained several specimens.

78. *Mycteroperca microlepis* Good and Bean (979). S. 846. Obtained one specimen.

79. *Epinephalus morio* (Cuv. and Val.), (983). S. 849. Red Grouper. One specimen reported by Dr. Yarrow.

38. Sparidæ.

80. *Orthopristis chrysopterus* (Lin.), (1026). S. 867, 868. Hog Fish. Very abundant.

81. *Hæmulon plumieri* Lac. (1046). S. 872. Black Grunt. Given by Dr. Yarrow, but thought by Jordan and Gilbert to be confused with the preceding.

82. *Stenotomus chrysops* (Lin.), (1063). S. 881. Scup. Not common.

83. *Diplodus rhomboides* (Lin.), (1064). S. 882. Pin Fish, Robin. Very abundant.

84. *Diplodus probatocephalus* (Walbaum), (1066). S. 883. Sheepshead. Abundant.

85. *Diplodus holbrooki* (Bean), (1067). S. 884, 885. Spot-tailed Pin Fish. Very abundant.

86. *Kyphosus sectatrix* (Lin.), (1069). S. 887. One specimen obtained by Jordan and Gilbert.

39. Sciænidæ.

87. *Pogonias chromis* (Lin.), (1084). S. 894. Sea Drum. Common.

88. *Sciæna chrysura* (Lac.), (1087). S. 896. Perch. Common.

89. *Sciæna ocellata* (Lin.), (1091). S. 898. Drum. "Rather common." Jordan and Gilbert.

90. *Liostomus xanthurus* Lac. (1095). S. 902. Spot. Very abundant.

91. *Micropogon undulatus* (Lin.), (1099). S. 904. Croaker. Very abundant.

92. *Menticirrus littoralis* (Holbrook), (1105). S. 908. Sea Mullet. The young in large numbers on Ft. Macon beach.

93. *Menticirrus saxatilis* (Bloch and Schneider), (1108). S. 907. Whiting. Reported by Dr. Yarrow as abundant in the fall.

94. *Menticirrus alburnus* (Lin.), (1109). S. 909. Whiting. Obtained a few specimens.

95. *Cyonoscion regale* (Bloch and Schneider), (1113). S. 915. Sea Trout. Common.

96. *Cyonoscion maculatum* (Mitch.), (1120). S. 917. Speckled Trout. Abundant.

40. Gerridæ.

97. *Gerres gula* Cuv. and Val. (1128). S. 920, 921. Obtained several young specimens.

41. Labridæ.

98. *Hiatula onitis* (Lin.), (1151). S. 942. Tautog, Oyster Fish. Common.

99. *Platyglossus bivittatus* Bloch (1159). S. 947, 948. One specimen obtained by Dr. Yarrow.

100. *Platyglossus maculipinna* (Müller and Troschel), (1161). One young specimen obtained by Jordan and Gilbert.

42. Ephippidæ.

101. *Chætodipteris faber* (Broussonet), (1197). S. 962. Porgee. Common.

43. Gobiidæ

102. *Gobius encæomus* Jordan and Gilbert (1226). S. 983*b*. Scallop Fish. Obtained about thirty specimens with considerable difficulty.

103. *Gobiosoma bosci* (Lac.), (1243). S. 993, 994. Obtained a few specimens.

44. Triglidæ.

104. *Prionotus scitulus* Jordan and Gilbert (1384). S. 1123. Slim Flying Toad. Two specimens taken by Jordan and Gilbert.

105. *Prionotus tribulus* Cuv. and Val. (1389). S. 1125. Common Flying Toad. Very common.

106. *Prionotus evolans* (Lin.), (1390). S. 1126. Flying Toad. Common.

107. *Cephalacanthus volitans* (Lin.), (1393). S. 1128. Flying Fish. Common.

45. Batrachidæ.

108. *Batrachus tau* (Lin.), (1419). S. 1149. Toad Fish. Very abundant.

46. Uranoscopidæ.

109. *Upsilonphorus y-græcum* (Cuv. and Val.), (1428). S. 976. Star-gazer. Two specimens obtained by me.

110. *Upsilonphorus guttatus* (Abbot), (1429). S. 977. Star-gazer. One specimen taken by Dr. Coues.

47. Blennidæ.

111. *Chasmodes bosquianus* (Lac.), (1439). S. 1153. Common. Several specimens obtained.

112. *Hypsoblennius punctatus* (Wood), (1445). S. 1156, 1156*b*. Abundant.

113. *Hypleurochilus multifilis* (Girard), (1448). S. 1157. Abundant.

114. *Hypleurochilus geminatus* (Wood), (1449). S. 1158. Abundant.

48. Lycodidæ.

115. *Zoarces anguillaris* (Peck), (1503). S. 1200. Eel-pout. Two specimens taken by Dr. Yarrow.

49. Ophidiidæ.

116. *Ophidion marginatum* De Kay (1525). S. 1219, 1220. One specimen seen by Dr. Coues.

50. Gadidæ.

117. *Phycis regius* (Walbaum), (1543). S. 1229. Codling. One specimen taken by Dr. Coues.

51. Pleuronectidæ.

118. *Bothus maculatus* (Mitch.), (1576). S. 1255. Plaice. Common.

119. *Citharichthys macrops* Dresel (1586). Proc. U. S. Nat. Mus. 1885. Obtained a few specimens.

120. *Citharichthys microstomus* Gill (1589). S. 1261. Not common.

121. *Etropus crossotus* Jordan and Gilbert (1590). S. 1296. Obtained a few specimens.

122. *Paralichthys dentatus* (Lin.), (1596). S. 1265. Flounder. Abundant.

123. *Paralichthys lethostigma* Jordan and Gilbert (1597). S. 1266. Flounder. Abundant.

124. *Paralichthys albigutta* Jordan and Gilbert (1598). S. 1267. Flounder. Common.

125. *Ancylopsetta quadrocellata* Gill (1601). S. 1270. Not common.

52. SOLEIDÆ.

126. *Achirus achirus mollis* (Lin.), (1635). S. 1299. Sole. Obtained a number of specimens.

127. *Aphorista plaguisa* (Lin.), (1637). S. 1301. Sole. Very common.

53. LOPHIDÆ.

128. *Lophius piscatorius* Lin. (1639). S. 1302. All mouth. A number of jaw-bones and a complete skeleton of the head were picked up at Cape Lookout. Small specimens taken with hook. W. K. B.

54. OSTRACIIDÆ.

129. *Ostracion trigonum* Lin. (1656). S. 1316. Trunk Fish. Two specimens obtained by Dr. Yarrow. Small specimens often taken in tow-net outside. W. K. B.

55. BALISTIDÆ.

130. *Monacanthus hispidus* (Lin.), (1664). S. 1322. Common Fool Fish. Very common.

131. *Alutera schœpfi* (Walbaum), (1667). S. 1326. Fool Fish. Common. Jordan and Gilbert.

56. TETRODONTIDÆ.

132. *Lagocephalus lævigatus* (Lin.), (1669). S. 1328. Smooth Puffer. Found by Dr. Yarrow.

133. *Tetrodon tugidus* Mitch. (1674). S. 1332. Swell Toad. Very common.

57. DIODONTIDÆ.

134. *Chilomycterus geometricus* (Bloch and Schneider), (1680). S. 1337. Swell Toad. Very common.

Additions to Former Lists.

The following twenty-one species, which were in my collection, do not occur in Dr. Yarrow's list, or in the one published by Jordan and Gilbert, and, as far as I have been able to learn, were for the first time recorded by me as occurring at Beaufort in a note in the J. H. U. Circular of October, 1885:

1. *Pristis pectinatus* Latham.
2. *Gambusia patruelis* (Baird and Girard).
3. *Conger conger* (Lin.).
4. *Tylosurus hians* (Cuv. and Val.).
5. *Hippocampus punctulatus* Guichenot.
6. *Querimana gyrans* Jordan and Gilbert.
7. *Menidia menidia* Lin.
8. *Elacate canada* (Lin.).
9. *Chloroscombrus chrysurus* (Lin.).
10. *Caranx latus* Agassiz.
11. *Caranx bartholomæi* Cuv. and Val.
12. *Nomeus gronovii* (Gmelin).
13. *Serranus philadelphicus* (Lin.).
14. *Serranus subligarius* (Cope).
15. *Mycteroperca microlepis* Good and Bean.
16. *Gobius encæomus* Jordan and Gilbert.
17. *Gobiosoma bosci* (Lac.).
18. *Upsilonphorus y-græcum* (Cuv. and Val.).
19. *Paralichthys lethostigma* Jordan and Gilbert.
20. *Etropus crossotus* Jordan and Gilbert.
21. *Etropus microstomus* Gill.
22. *Citharichthys macrops* Dresel.
23. *Lepidosteus osseus* Lin.

Note.—Since the above has been in type Dr. Jordan has published in Proc. U. S. Museum, 1886, *Notes on Fishes of Beaufort*, in which he states that *Hippocampus hudsonius* De Kay, of Synopsis Fishes N. A., came from Beaufort.

Greencastle, *July* 31, 1886.

THE STRUCTURE OF CUNOCTANTHA OCTONARIA IN THE ADULT AND LARVAL STAGES.

By H. V. WILSON, Johns Hopkins University.

With Plates I, II, and III.

This medusa, which spends its larval existence in the bell-cavity of *Turritopsis nutricola*, has been described and figured by McCrady[1] and Brooks.[2] The metamorphosis the larva undergoes while in the *Turritopsis* has been also fully described from surface views of the living animal by these authors. The following paper, then, must necessarily contain a certain amount of repetition, but the additional points I have made out, by means of sections, will at any rate be of some use in completing our knowledge of *Cunoctantha*, so far as the structure of the adult and larva is concerned. The development of the egg has not yet been followed.

My material was collected during the past summer at Beaufort, N. C., where *Turritopsis* with parasitic *Cunoctanthas* was common from July 4 to September 1, when I left. As a hardening agent I have used osmic acid exclusively.

Anatomy of the Adult.

A diagrammatic view of the animal from above is given in Fig. 1, and a radial section in Fig. 2. On the left the section is through a tentacular radius, on the right through an interradius. The margin of the umbrella is indicated by the line *u* in Fig 1. The velum, *v*, is represented in the figure as stretched out flat. The nerve-cord, *n*, consists of eight bold curves connected in pairs by means of the peronium, *P*. In the interradius the cord is at the margin, but from there it curves dorsally, meeting its fellow-segment at a point just peripheral to the peronium. There is then for a very short distance a single nerve-tract which enters and makes part of the peronium, as shown in Figs. 1 and 17. The peronium is an ectodermal protuberance beneath the base

[1] Gymnophth. Charleston Harbor, 1857.

[2] Life-History of the Hydro-Medusae. Mem. Boston Soc. Nat. His., 1886.

of the tentacle, with the epithelium of which it is continuous, Figs. 1, 2 and 17. Seated on the nerve-cord are the sense-clubs or marginal bodies, three to each of the eight segments. Running dorsally from each sense-club there is the narrow stripe of thickened ectoderm, provided with nettle-cells, called by Haeckel the otoporpa, Figs. 1, 2 and 5, *O*.

The mouth is irregular in shape. Radiating from the stomach, *G*, are the eight gastric sacs, *G. S.*, through one of which the left half of the section, Fig. 2, is cut, while the right half lies between two sacs. The ectoderm of the floor of each sac is transformed into the genital elements, Figs. 2 and 17, *G. e.* Projecting into the umbrella-jelly just above each sac is the inward prolongation of the tentacular endoderm, known as the root of the tentacle, *R*. A membrane of flattened cells, the endodermal or vascular lamella, continuous with the endoderm of the stomach and sacs, stretches from the margin of the gastric system to the nerve-cord, *n*. The outline of the umbrella-jelly is likewise determined by the nerve-cord, and in this way the umbrellar surface becomes very noticeably lobed.

The space marked *v'* in Fig. 1 shares in forming the dorsal surface, but contains neither endodermal lamella nor umbrella-jelly. A section across this area vertical to the surface and transverse to the radius is given in Fig. 6. The jelly stands up on either side, and the area itself is a simple ectoderm membrane possessing exactly the same structure as the velum. This is seen from a comparison of Fig. 6, *v'*, with Figs. 3 and 5, *v*. A radial section through this region (perradial) is shown in Fig. 17. The area in question is in fact a direct continuation of the velum upon the dorsal surface, and functions as an interlobular web. I have called this area the ascending velum. (In the other *Narcomedusae* described, except in the family *Peganthidae*, the place of this ascending velum is occupied by a narrow stripe of ectoderm several layers thick, containing nervous elements and nettle-cells, and called by Hertwig the radial string, by Haeckel the peronium.) The true outline of the umbrella in *Cunoctantha* is therefore indicated by the nerve-cord, *n*, the apparent margin, *u*, being merely the bend between the ascending and horizontal velum. The outline is evidently lobed, though the division into lobes is imperfectly concealed by the part the velum plays in forming the umbrellar surface.

The nerve-cord, sections of which are given in Figs. 3, 5 and 6, is the umbrellar cord. The subumbrellar cord is so slightly developed that it is only recognizable in sections through the region of the sense-organ, Fig. 5, *S. Co.* The sense-club is seated on the umbrellar cord, which at this point forms a rounded elevation, roughly a hemisphere, the auditory cushion or *hörpolster* of Hertwig. The section, Fig. 5, was thin enough to include but a single layer of the cells composing the cushion, and therefore an approximate idea of the number of sense-cells in the cushion may be got from the figure. The shape of the sense-cells could not with certainty be ascertained from sections. They appeared, however, to resemble the elongated spindle-cells described by Hertwig in *Cunina lativentris*, *Æginopsis*, etc.[1] Between the sense-cells I observed several nettle-cells. Running up from the cushion is the otoporpa, *O.* The cells composing it form a single layer and are of a cubical shape, standing out prominently against the surrounding flat epithelium of the umbrella-surface. Comp. Figs. 5 and 3. Scattered amongst the cubical cells are nettle-cells, *n. c.* The connection of the sense-club with the body is formed in the manner described by Hertwig. The supporting layers of the umbrella and sub-umbrella unite to form a thread, which pierces the cushion and then expands into the supporting layer of the club. The mature sense-club in this medusa has two crystals. The club represented in Fig. 5 is probably backward in its development. In Fig. 3 is shown a section of that portion of the nerve-cord which lies between two sense-organs. The nerve-epithelium cells are columnar. Neither here nor in the ascending portions of the cord was I able to observe nettle-cells, though they may be present in small number. Haeckel claims that in the *Narcomedusae* there is present a nettle ring comparable with that of the *Trachomedusae*, only smaller: "Er (Nesselring) besteht aus einem dünnen Streifen modificirter Nesselzellen und ist von flimmerndem Sinnes-Epithel überzogen" (System d. Medusen, p. 307). Evidently there is nothing of the kind in *Cunoctantha*, and the structure is equally absent from the nerve-rings of the three *Narcomedusae* described by the Hertwigs (*l. c.*). In Fig. 6 the ascending portions of the nerve-cord are cut as they move towards the peronium. The histological

[1] Nervensystem und Sinnesorgane d. Medusen. 1878.

structure is like that of the cord at the margin, but the cord itself is larger. In my figures of the nervous system I have not represented the nerve-epithelium cells as ciliated, because the reagents used destroyed every trace of the cilia.

The two nerve-tracts shown in Fig. 6 run up, unite and form the peronium, Figs. 1 and 17. The great mass of this structure, which is to be regarded as the much-enlarged uppermost limit of the nerve-cord, is not nervous. The nervous elements are restricted to a small space situated in the angle between the perohium and the ascending velum, Fig. 17, *n*, and in the radius bisecting the peronium. The structure of the organ is essentially that of the nettle-ring found in *Trachomedusae;* and if, as Haeckel thinks, the *Narcomedusae* have been derived from the *Trachomedusae*, it is to be regarded as the remnant of a ring that once accompanied the nerve-cord throughout its length. The characteristic element of the peronium is the modified nettle-cell, an oval cyst with a double contour, often of considerable size. I was unable to make out threads in any of the cysts, though many of the similar elements in the nettle-ring of *Trachomedusae* contain threads. Each cyst arises in the interior of a formative cell, which, as the Hertwigs have shown, remains permanently round the cyst. The modified nettle-cells are very numerous, and lie in a kind of matrix formed of very small and much-compressed cells. In these cells no doubt fresh cysts arise. The small group of polygonal cells just internal to the nerve-cord in Fig. 17 belong to the class of matrix-cells, but happen to be much larger than the rest. Owing to its excessive growth, the peronium has in a manner absorbed the epithelium-cells on the under surface of the base of the tentacle. The intimate connection of these cells with the peronium, their elongated, somewhat irregular shape, and general sickly appearance, are shown in Fig. 17.

Covering the peronium is a very thin membrane of flattened epithelium-cells continuous with the epithelium of the tentacle above, the velum beneath, and the umbrella at the sides. The general shape and position of the peronium are indicated in Fig. 1. It lies beneath, but also embraces the sides of the tentacle, or, in other words, the tentacle lies in a shallow depression of the peronium. The latter on both sides pushes its way into the jelly, so that only the middle portion, that directly beneath the

tentacle, is exposed to the exterior. The whole structure indicates that the peronium is not an organ of defense, but of support, which, considering the position and weight of the tentacles, must be of great importance to the animal.

The structures known as the tentacular roots, Fig. 17, *R*, are probably of the same physiological use as the peronia. Haeckel describes the root as covered by a structureless membrane. This membrane, however, exhibits here and there a nucleus, and, as the development shows, represents a double layer of ectoderm. One of these layers is continuous with the epithelium of the umbrella, the other with the epithelium of the tentacle. The root is therefore, in a morphological sense, outside of the jelly.

Hitherto I have not mentioned the canal system. Sections prove that no canals exist. In Fig. 5 the vascular lamella is shown in the radius of a sense-organ. It here ends in four cells considerably larger than the other cells of the lamella. It is only in the region of the sense-organs that there is this enlarged periphery, the lamella having elsewhere but a single layer of cells throughout its extent. This is shown in Figs. 3 and 6, where the vascular lamella abuts against the nerve-cord, the terminal cell being somewhat larger than the average. If the canals did exist at one time, and afterwards closed up, it is only in the region of the sense-organs that they can be said to have left traces of their former presence. As will be seen, there is in the individual development no formation of canals which subsequently atrophy. On the contrary, the vascular lamella is quite uniform during the larval stages, and it is only when the medusa has assumed the likeness of the adult, and is of considerable size, that the periphery of the lamella becomes enlarged in the particular places mentioned.

The Larval Stages.

The youngest larva observed by Brooks or McCrady is a hydra-like organism minus the stalk, with a much-elongated proboscis, and a pair of tentacles which point dorsally from the edge of the ab-oral surface, Fig. 7. In a single instance I observed a younger stage, shown in Fig. 15. In shape it was an elongated rod slightly swollen at one end. It had neither mouth nor tentacles, but the two layers were well defined.

Two more tentacles develop in the bi-tentaculate larva at right angles to the plane of the first pair. At this stage or a little later begins the formation of dorsal buds, as described by the above authors. The first step towards the formation of the medusa is the outgrowth of the ab-oral portion of the body into four lobes, one between each pair of tentacles, Fig. 8. These lobes contain prolongations of the gastric cavity and lie in a horizontal plane. The tentacles have retained their primitive position, but owing to the growth of the lobes have also come to lie in the interlobular notches. A radial section through the tentacle on one side and the middle of a lobe on the other is shown with tolerable accuracy in Fig. 10, though this figure actually belongs to a slightly older stage. The ectodermal thickening just beneath the tentacle constitutes the only point of difference. It is absent in the younger stage. The point to be noticed here is that the outgrowing flange, which afterwards makes most of the medusa-bell, does not arise as a continuous rim at the edge of which are situated the tentacles, which later travel dorsalwards: it is, on the contrary, made up of four disconnected lobes, between which lie the tentacles.

Fig. 4 gives a view of the ab-oral surface of my next stage. The four lobes have become eight, though there are still but four tentacles. The endoderm at the points (t') where the four secondary tentacles will arise is probably already modified, but of this my sections do not assure me. At all events the tentacle is not yet formed. What has evidently occurred in the transition from the four-lobed stage to this is the stoppage of growth, both of endoderm and ectoderm, at the middle point of the periphery of each lobe, thus leading to the formation of two from each of the four primary lobes. Fig. 10 shows a combined perradial and interradial section through this stage. There is nothing that can as yet be called an interlobular web, but the foundation of such is laid in the thickening of ectoderm (x) just below the tentacle. This thickening is the first indication of the future velum, or rather of that part of it which serves as an interlobular web, and which in the adult I have called the ascending velum. It is not until a considerably later stage that the velar outgrowth appears in the interradii, where of course it only forms the horizontal velum. In the ectodermal thickening mentioned, the supporting

layer has grown out between the cells, which in turn have become columnar, and so inclined as to rest with their bases upon the supporting layer. The gastric epithelium is no doubt ciliated, though the reagents have left but a few traces of the cilia. The dark bodies scattered through the endoderm of this and succeeding stages I believe to be algæ. At least they have a more or less regular shape, and here and there show signs of division. They are present in the adult, but in proportionately much smaller numbers. The osmic acid unfortunately makes them very opaque, and in examining the living animals I did not notice them. I am consequently in doubt whether they be algæ or merely patches of pigment.

Fig. 9 represents a section through two opposite lobes of a stage in which the secondary four tentacles have appeared, but are very small. The cavity of one lobe is open, that of the other is closed. The endoderm of the latter retains its two-layered character, but this will soon disappear, giving place to the single layer of endoderm-cells known as the vascular lamella. At the edge of the closed lobe the future velum is barely indicated. Here, as in the tentacular radius of the preceding stage, the supporting layer forms an outgrowth, and the ectoderm-cells begin to incline their long axes at right angles to it.

Fig. 11 is through the tentacular radius of the same larva. The velar outgrowth begun in the preceding stage, Fig. 10, is more pronounced, especially on the upper side of the supporting layer. The thickening here is the first appearance of the peronium, the cells of which are as yet undifferentiated, there being no sign of the cysts. The size of the peronium makes it possible at this stage to speak of an interlobular web, though this web only very partially fills out the space between two lobes. The gastric epithelium of the last stage was tolerably uniform throughout the larva, the epitheliar surface being smooth and provided with a thin cuticle, Fig. 10. In this stage, Fig. 9, there is no evident cuticle, and the cells of the ab-oral swollen portion of the larva differ from those lining the cylindrical proboscis. The former are very long, much vacuolated, and contain many granules. The epitheliar surface is no longer smooth, but raised into irregular hillocks. The epithelium of the proboscis retains its former size and smooth bounding surface, but has a

number of very clear and large vacuoles. The cells are evidently losing their protoplasm, and offer a transition to the epithelium lining the proboscis of the next older larva, Fig. 16. The length of the proboscis has been slowly diminishing, and this diminution continues until there is nothing left save a thickened vein round the mouth. In Fig. 13 one of the secondary tentacles is shown. The section does not lie in the radius of the tentacle, but cuts a lobe on the right hand. The cells of the gastric epithelium are quite large, and between them and the endoderm of the tentacle there seems not to be that easy transition shown in the developing sense organ, Fig. 19. This last figure is a surface view from a larva slightly older than the one from which the sections, Figs. 11, 9, 13, are taken.

The larva next to be described has eight tentacles, about equal in size. A radial section is given in Fig. 16. The lobes have grown very much. In life they stand out in nearly a horizontal plane, but when the animal is killed they contract into the pendent position shown in the figure. The interlobular web has completely filled up the notches, and has also bent in to form the horizontal velum. The horizontal velum is continuous all round the medusa, the ectodermal outgrowth having progressed in the radius of the sense-organ as well as in the tentacular radius. The cavities of the lobes have completely disappeared, their endoderm having gone to form the vascular lamella. The gastric cavity hence is circular in outline when viewed from the dorsal surface. The cells of the vascular lamella are cubical and form a single layer. The sense-organ projects as a prominent papilla, the endodermal axis of which is still continuous with the lamella. In the terminal endoderm-cell the crystal has begun to form. The cells of the peronium have manifestly altered. The external cells are flattened and nucleated. In many of the internal cells nuclei are to be seen, but in others the nucleus has disappeared and a small cyst with a double contour is found in the cell. Occasionally both the cyst and nucleus are found in the same cell, but the latter is usually lost when the cyst-formation begins. The auditory cushion at the base of the sense-organ is quite evident, especially on the upper side of the club. The cells are here very long and slender. Elsewhere the nerve-tract is not so prominent, but at the edge of the lobes all round the

medusa the ectoderm-cells are somewhat more columnar than in other places.

The gastric epithelium has made another step towards differentiation. The much-elongated, vacuolated cells are confined to a more restricted area than in the preceding stage. This area, as the proboscis shortens and the floor of the stomach comes to lie horizontally, forms the epithelium of this surface. The dorsal endoderm of the larva has lost its vacuolated character and has acquired a smooth surface. The cells henceforward gradually change from a columnar into a cubical or more flattened shape, and form the dorsal epithelium of the adult stomach. A transverse section of the proboscis lies in the gastric cavity. The protoplasm of the ectoderm-cells is much vacuolated, but in the endoderm-cells is reduced to a small heap, in which lies the nucleus. The ectoderm over the dorsal surface of the medusa begins to flatten from this stage onwards.

The umbrellar jelly has begun to develop, but it will be noticed that just at the dorsal limit of the tentacular insertion, Fig. 16, the ectoderm remains separated from the endoderm only by the thin supporting layer. In a somewhat older stage, Fig. 14, the umbrellar jelly is much thicker, and in its growth has caused the proximal portion of the tentacle to be included within the body of the medusa. This portion of the tentacle is separated from the jelly by a double layer of ectoderm. In Fig. 12 the included portion of the tentacle has begun to bulge out towards the jelly, and thus to commence the formation of the root. The double layer of ectoderm no longer exists as such, but appears as a nucleated membrane covering the developing root, as in the adult medusa.

In Figs. 14 and 12 the peronium has continued its development, the cysts increasing in size until in sections nothing is to be seen of the cell in which each was originally formed. Certain of the undifferentiated cells of the larval peronium remain to form the small matrix-cells of the adult, while the superficial flattened cells continue to grow flatter and form the delicate epitheliar membrane of the fully-developed peronium.

When the young medusa leaves its host, it is essentially like the adult except in one important particular (Brooks, *l. c.*). The outline of the gastric cavity is a circle, running through the

insertion-points of the eight tentacles. Only after a free swimming life of some duration, and a very considerable increase in size, does the endodermal fusion take place in the interradii by which the gastric sacs are formed.

Haeckel's idea of the *Narcomedusae*[1] is that they are descended from the *Trachomedusae*, and that the chief modifying cause has been the migration of the tentacles from the umbrellar margin dorsalwards. The tentacles when they left the margin remained connected with the nerve and nettle-ring, and, the distance increasing, this connection took the form of the narrow stripe of thickened ectoderm, the peronium or radial string which runs from the base of the tentacle to the marginal ring. Moreover, the circular canal was carried dorsally by the tentacles in their several radii. It thus became divided into marginal segments and double canals. The latter course along the sides of the radial string and put the marginal segments in communication with the gastric cavity above. In one family, however, the *Peganthidae*, the tentacles carried with them the umbrellar margin in such a manner as to divide it into a number of lobes, between which lay stretched the velum. The nerve and nettle-ring and the circular canal here formed a number of curves connected at the bases of the tentacles, where the segments of the canal open into the gastric cavity, and those of the nerve and nettle-ring usually form a swelling, the reduced peronium.

Now, the history of *Cunoctantha* shows that the four primary tentacles, in the ontogenetic development at least, do not attain their ultimate position by a migration from the margin of the umbrella. As is expressed in the diagram, Fig. 18, they retain their original position, which comes to be at a distance from the margin for two reasons: First, the outgrowth of intertentacular lobes; and, second, the growth of the velum, which fills up the interlobular notches, thus making whole the umbrellar surface, and then bends in to form the horizontal velum, making in this way a new umbrellar edge. The case of the four secondary tentacles is slightly different. Though they themselves never lie at the edge of the flange, the endoderm from which they ultimately come does; and this may be considered as if it were the tentacle itself. This endoderm retains its position, the secondary lobes

[1] Das System d. Medusen. 1879.

growing out on both sides, as in the diagram. Thus for some time the physiological as well as morphological margin of the umbrella is eight-lobed, and the tentacles are at the margin, in the interlobular notches. Only later, by the growth of the velum, are they separated from it.

Though the ontogeny of *Cunoctantha* does not, in this matter of the tentacles, confirm Haeckel's theory in a manner so straightforward as to admit of one construction only, the differences appear almost certainly to be of secondary origin, and due to the early development of the tentacles.

Haeckel divides the *Narcomedusae* into four families, characterized by the presence or absence of otoporpae and the shape of the gastro-canal system. The *Cunanthidae* and *Peganthidae* have otoporpae. The *Æginidae* and *Solmaridae* have not. All but the *Solmaridae* have the peculiar peripheral canal-system, and in the *Solmaridae* traces of this system are left. The *Cunanthidae* have pernemal gastric sacs—*i. e.* one in the radius of each tentacle. The *Æginidae* have internemal sacs, two between each two tentacles. The *Solmaridae* may have either pernemal or internemal sacs, or only a simple circular stomach. The *Peganthidae* have a simple stomach without sacs.

Now, it will be seen that it is quite impossible to assign *Cunoctantha* to any of these families. Haeckel places it in the *Cunanthidae*, but it has no canal-system at all, and hence from this point of view must enter the *Solmaridae*. The pernemal gastric sacs belong alike to the *Cunanthidae* and some *Solmaridae*, and can have no weight. But the presence of otoporpae throws it out of the *Solmaridae*. Again, in the most important respect of all—in the shape of the true umbrellar edge—it belongs to neither of the last-mentioned families, but to the *Peganthidae*. Haeckel's classification is therefore in so far unsatisfactory as no place is found for *Cunoctantha*. Metschnikoff finds a similar difficulty as regards *Cunina proboscidea*,[1] of which there are two forms, one probably produced from a fertilized egg, and the second produced from spores or by budding. The first form has otoporpae, a canal-system—in fact, all the characteristics of the *Cunanthidae*. The second has neither otoporpae nor canals, and is a true *Solmarid*.

[1] Embryologische Studien an Medusen. Wien, 1886.

INDEX TO LETTERS USED.

a. c. Auditory cushion.
b. Bud.
ec. Ectoderm.
en. Endoderm.
e. l. Endodermal lamella.
G. Stomach.
G. S. Gastric sac.
G. e. Genital epithelium.
m. Mouth.
n. Nerve-cord.
mu. Muscle-fibres.
n. c. Nettle-cell.
O. Otoporpa.
P. Peronium.
R. Root of tentacle.
S. C. Sense-club.
S. Co. Subumbrellar cord.
u. e. Umbrellar ectoderm.
s. e. Subumbrellar ectoderm.
u. Margin of umbrella.
T. Tentacle.
t'. Points where secondary tentacles will appear.
v. Horizontal velum.
v'. Ascending velum.
x. Commencing velum.

EXPLANATION OF THE FIGURES.

The supporting layer in all the figures is represented by a thin black line.

The sections were drawn with the camera and Zeiss lenses. Plate I was reduced slightly more than one-half. Plates II and III were reduced one-third.

FIG. 1.—Diagrammatic surface view of the adult medusa. The velum is spread out flat. The middle tentacle is removed to show the peronium.

FIG. 2.—Section of the adult medusa, through the tentacle on one side and the sense-club on the other.

FIG. 3.—Adult. Radial section through nerve-cord, between two sense-organs. Velum vertical. F. Oc. 4.

FIG. 4.—Ab-oral surface of larva with eight lobes and four tentacles.

The larva had a dorsal bud not shown in figure. Living animal. D. Oc. 4.

FIG. 5.—Adult. Radial section through sense-club and auditory cushion. Velum hangs down. F. Oc. 4.

FIG. 6.—Adult. Section perpendicular to umbrellar surface and across radius of tentacle. It strikes the ascending velum and the ascending portions of the nerve-cord. Velum is not figured throughout its whole width. F. Oc. 4.

FIG. 7.—Bitentaculate larva. Optical section of living animal. D. Oc. 4.

FIG. 8.—Larva with four tentacles and four lobes. From living animal. D. Oc. 4.

FIG. 9.—Radial section of larva with eight lobes and eight tentacles. Section is through a lobe on each side. D. Oc. 4.

FIG. 10.—Radial section through larva of same age as Fig. 4, through a tentacle on one side and the middle of a lobe on the other. Two dorsal buds present. Lower part of proboscis unfigured. D. Oc. 4.

FIG. 11.—Section through tentacular radius of same larva as Fig. 9. D. Oc. 4.

FIG. 12.—Section through tentacular radius of a young medusa which has just left its host. D. Oc. 4.

FIG. 13.—Secondary tentacle of same larva as Fig. 9. The section is not in the radius of the tentacle, but cuts the side of a lobe, as shown in the right-hand part of figure. F. Oc. 4.

FIG. 14.—Section through tentacular radius of a larva slightly older than Fig. 16. The proximal portion of the tentacle is included within the medusa-body, and is separated from the jelly by a double layer of ectoderm. D. Oc. 4.

FIG. 15.—Larva without mouth or tentacles. D. Oc. 4.

FIG. 16.—Radial section of larva in which umbrellar jelly has just appeared. The section cuts a sense-organ on the left and a tentacle on the right. A transverse section of the proboscis lies in the gastric cavity. D. Oc. 4.

FIG. 17.—Adult. Radial section through the tentacle. D. Oc. 4.

FIG. 18.—Diagram to illustrate positions occupied by the tentacles during the several changes of the umbrellar margin. T' is the primary, T'' the secondary tentacle. The line I represents the outline of the ab-oral surface of a larva without lobes; the line II that of a larva with four lobes; the line III that of a larva with eight lobes.

FIG. 19.—Very young sense-club. Surface view of the tip of a lobe of a larva slightly older than Fig. 9. Osmic preparation. F. Oc. 4.

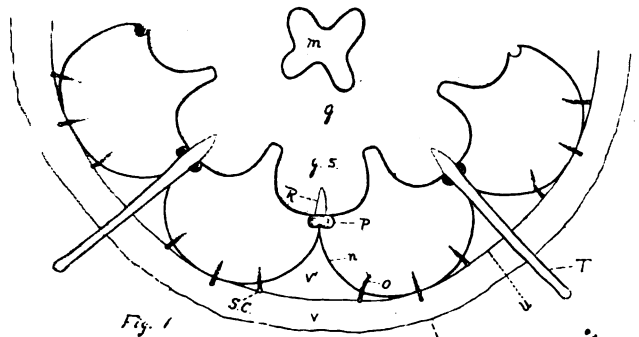

Fig. 1

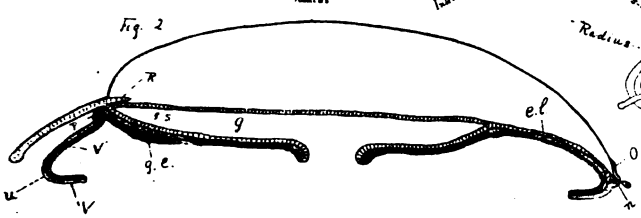

Fig. 2

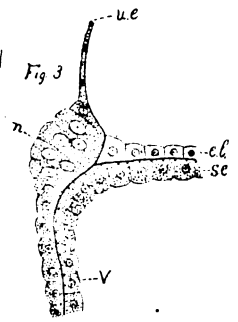

Fig. 3

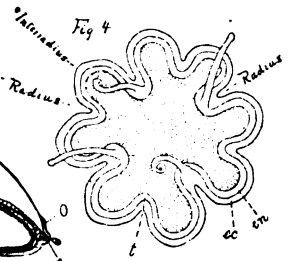

Fig. 4

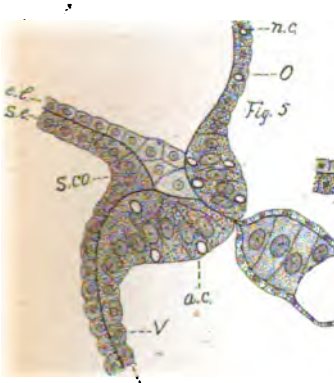

Fig. 5

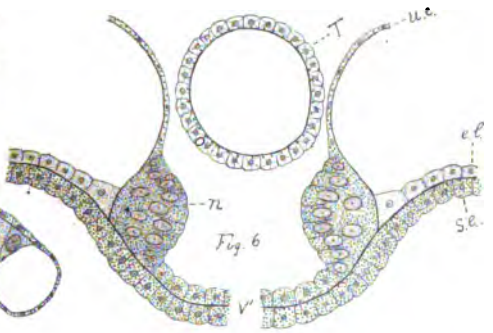

Fig. 6

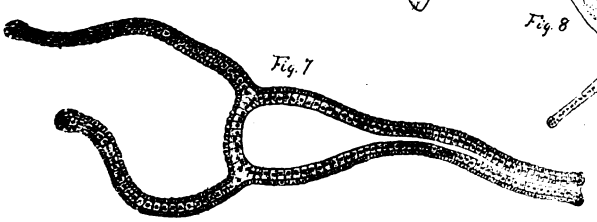

Fig. 7

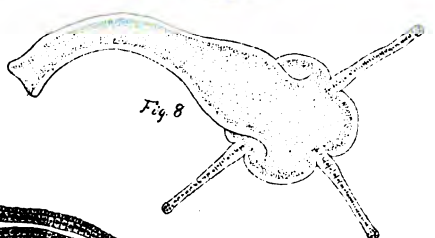

Fig. 8

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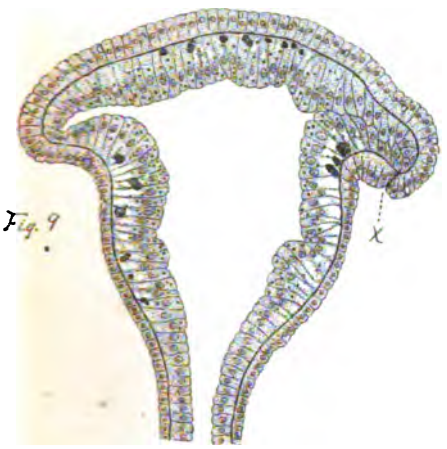
Fig. 9
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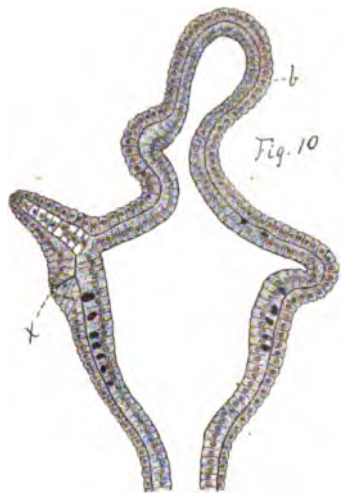
b
Fig. 10
x

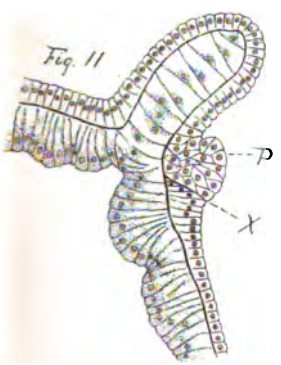
Fig. 11
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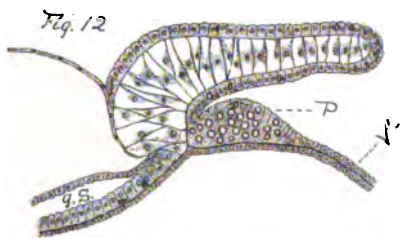
Fig. 12
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g.s.

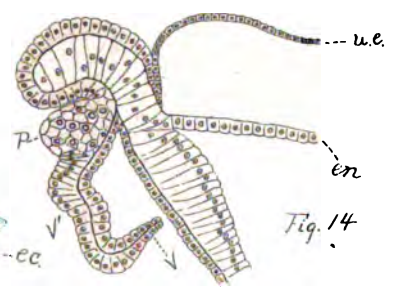
u.e.
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Fig. 14

Fig. 13
ec
e.n

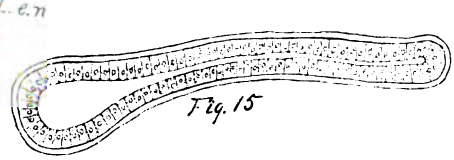
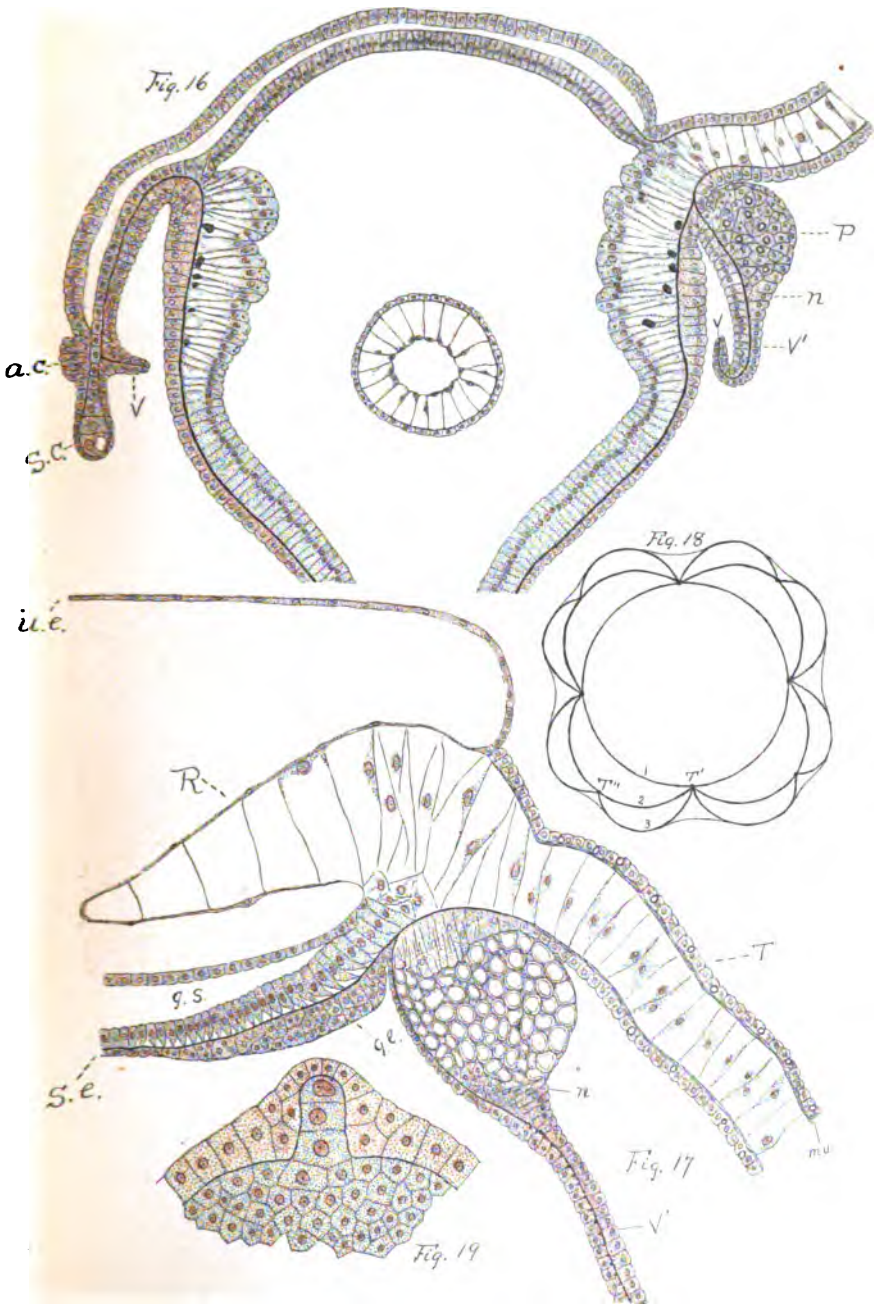


Fig. 15



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SOME OBSERVATIONS ON THE LAWS OF MUSCULAR STIMULATION AND CONTRACTION, MADE ON THE MUSCLES OF THE TERRAPIN.

By GEORGE T. KEMP, Ph. D., Fellow by courtesy of the Johns Hopkins University. With Plates VII, VIII, and IX.

In spite of the fact that for the last twenty-five years the subject of nerve and muscle physiology has engaged the attention of many of the most eminent physiologists, there is scarcely a field in which more conflicting results have been obtained, or from which a richer harvest awaits improved methods of investigation. The great need is to get a nerve or muscle which will retain its normal vitality and properties for a considerable time after removal from its place in the body.

Up to the present time, by far the greatest part of the work done in this field has been carried on in Germany, on the nerves and muscles of frogs, and the readiness with which frogs' nerve and muscle undergo change has been constantly noted by those engaged in these researches.

Terrapin's muscle is a tissue which lends itself admirably to investigation, and which is in great measure free from the disadvantages attending the use of the corresponding tissue of the frog. This is especially true in the case of the neck retractors of the terrapin, which run from the skull and foremost cervical vertebræ back to the posterior dorsal and the lumbar vertebræ, thus giving a stretch of pure muscle the fibres of which are almost parallel for several inches. The attachment of these muscles is, furthermore, such as to admit of their being removed without injury to the muscle. The vitality of the terrapin's neck retractors is astonishing. I have frequently kept them working for three or four hours before they showed signs of exhaustion. In one case I worked a muscle at different intervals for seven hours, in the meantime having obtained over forty recorded curves from it, and when finally discarded it could still respond to stimuli, although its sensitiveness had diminished considerably. This makes a striking contrast when compared with the results

of other investigators who have used the muscles (especially the thin ones) of the frog.

Aeby[1] has shown that the rate of propagation of the contraction wave may sink more than 50 per cent. in a few minutes in a thin excised muscle like the sartorius. Valentin,[2] to avoid this error, devised an ingenious method of working with the sartorius *in situ*, and even then found that after an hour the rate had fallen from 4.28 meters to 1.7–.7; and four and a half hours after the beginning of the experiment the rate was found to be .43 to .36 meters.

As terrapins can readily be procured in Baltimore, and as the Biological Laboratory here is equipped with many facilities for research on the physiology of nerve and muscle, I determined to test the adaptability of the terrapin's muscles to this kind of work, and began a series of experiments to find whether curarized muscle obeyed Pflüger's law for nerves. I had also intended to compare the rate of propagation of the stimulus wave in curarized muscle, as given by the wave of contraction, with the rate of propagation of the negative variation. The values of the rate of progression of the stimulus wave, as determined by these two methods, have never been found to agree to within 30 per cent. The result of the many careful experiments of Bernstein gives as a mean: Neg. variation : rate of progression :: 2.9 : 3.8 or :: 1 : 1.31, a variation of 31 per cent.

What Bernstein calls rate of progression of the contraction wave is really the rate of progression of the stimulus wave, determined by the contraction. Hence there is a variation of 31 per cent. in the same value, as determined by two different methods, which is hardly satisfactory.

The first part of the investigation I was able to accomplish successfully, but the second part I was unable to take up, as the catching of terrapins is limited by law to a certain season, and that season had expired before I was able to go on with the rheotome work. I had hoped to continue this investigation, and therefore delayed this publication, but as there is no prospect of my being able to resume this research in the near future, I

[1] Untersuch. über die Fortpflanzungsgeschwindigkeit der Reizung der quergestreiften Muskelfaser, p. 36. Braunschweig, 1862.

[2] Pflüger's Archiv, Vol. IV, p. 117.

publish the work as far as completed, as some results of interest have already been obtained.

Method of Experiment. Preparation of the Muscle.

To prepare a muscle for an experiment I proceeded as follows: An opening was made in the plastron of the terrapin just over the heart. At this point there are no muscular attachments. When the piece of plastron is taken out, the heart, lying in its pericardial sac, is directly exposed. The pericardium is now cut through, and the tip of the ventricles not too tightly held by a pair of forceps, while the needle of a fine hypodermic syringe is pushed through the ventricular wall slightly to one side of the apex, and from 8 to 10 minims of a one per cent. solution of curare in .75 per cent. solution of NaCl is injected directly into the ventricle. It is necessary to inject the curare directly into the circulation, for absorption is so slow in the terrapin that three times the amount of curare mentioned, when injected subcutaneously, has little or no effect even after eight hours.

When properly done the needle of the syringe may be pushed through the wall of the ventricle without the loss of more than a drop or two of blood. To accomplish this, it is best, after injecting the curare, to retain the apex of the ventricle between the forceps while the heart is making three or four beats, and then release it at the end of systole.

Within 20–30 minutes the animal is completely under the influence of the curare. To assure myself of this I did not rely on the pinching reflex, but isolated a nerve on the back of the thigh and stimulated it with a tolerably strong interrupted current. When the muscles of the leg no longer responded to this stimulus I knew that the animal was completely under the influence of the drug. Meanwhile the animal was pithed and its brain destroyed. The cerebral cavity was stuffed with cotton to prevent hemorrhage. The plastron was now removed and the skin cut loose from the carapace, along its whole line of insertion posterior to the bridge joining the plastron to the carapace. The symphysis pubis was next divided, and the attachment of the pelvis to the carapace broken by running a carpenter's gouge along the median line of the carapace. The precoracoidal symphysis was next cut, together with the plate of cartilage uniting the scapulæ,

and the two halves of the shoulder girdle were forced apart as far as possible.

If now the hind legs be drawn firmly and steadily forward they will carry with them the pelvis and peritoneum with its enclosed viscera, and leave the large neck retractors exposed.

The neck retractors are not single muscles, but are composed of several groups which are attached to the sides of the bodies of the vertebræ, in the cervical region anteriorly, and in the lower dorsal and lumbar regions posteriorly. As many of the groups as desired may be used. The best way to separate the muscles from their posterior attachments is to split the vertebræ along the median line with an arthrotome, and then, with a carpenter's gouge, to dig out a piece of the carapace with which are united the pieces of vertebræ to which the muscle is attached. As the cervical vertebræ are not fused with the carapace it is only necessary to split them and separate them with a scalpel from all attachments.

One prominent group of muscles belonging to the neck retractors is attached to the skull by a ligament which can be traced as far forward as the palatine bones, but the tendon of this group can easily be detached from its connections. When it is advisable to use only one group of the retractor muscles, this is the best adapted for the purpose.

By following carefully these methods there results little or no injury to the fibres. The greatest chance for injury comes in while splitting the vertebræ and severing their connections with adjacent parts, but after some practice this can be accomplished in a very few minutes; one end of the muscle being bathed in the natural exudation from the tissues, and kept from drying by being covered with viscera and peritoneum, while the other end is being operated on. The danger of producing changes in the muscle by subjecting it to abnormal conditions is thus reduced to a minimum. Care must also be taken to leave slightly more bone at each end than that to which the muscle is attached, in order that a string may be tied to it without injuring the fibres.

Part of the apparatus employed was a modification of that used by Bernstein,[1] in his work on the rate of propagation of the

[1] Untersuchungen über den Erregungsvorgang im Nerven und Muskelsysteme. Heidelberg, 1871.

wave of contraction in muscle; the rest is an arrangement of my own.

The muscle was placed in the moist chamber (Fig. 1, *A*) on a glass plate (Fig. 1, *B*), across which platinum wires were stretched at known distances apart, and so arranged that any one of them could be made the anode or cathode respectively for any given stimulus. A piece of strong thread was tied to the fragment of bone at each end of the muscle. One thread was fastened firmly, while the other passed through a hole in the bottom of the moist chamber and suspended the weight by which the muscle was stretched. On the muscle, near one end of the plate, was laid a narrow strip of glass (Fig. 1, *s*), drawn out to a thinness just sufficient to make it raise the weight of the recording lever without bending and so causing its own elasticity to influence the curve. This strip was kept in position by four pins (Fig. 1, p, p', p'', p'''). To each end of the strip was fastened a piece of silk; the two pieces were passed through a large hole in the bottom of the moist chamber and brought together below, thus making an inverted stirrup, of which the glass strip was the base. From where the strings were joined below a single piece of silk ran down to support a small ebonite pulley (Fig. 1, *P*), over which passed a piece of silk, connected at one end with the recording lever, while at the other it wound on the axis of a screw (*s'*) by which it could be wound up or let out and the recording lever raised or lowered accordingly.

This arrangement of the pulley is the same as that used by Bernstein. It is very convenient, as it doubles the lift of the lever and admits of easy adjustment.

One objection that has been urged against the apparatus of different observers is, that a considerable mass would have to be set in motion before the curve began to be recorded, and the momentum of this mass would tend to prolong and alter the true curve.

To obviate this is of great importance, and the apparatus employed was arranged with special regard to this point.

To a Foster's adjustable lever attachment (Fig. 1, *F*) was fastened a common straw, making the whole length of the lever about 25 centimeters. To the end of the straw was fitted a cap of writing paper, to which a piece of extremely thin aluminium

foil was soldered by a thin film of sealing wax. This piece of foil was trimmed to a point which recorded the curve.

I cannot speak too highly of aluminium foil for this purpose. When of the right thickness it has just the proper spring to keep the point sufficiently pressed against the plate of the myograph, and at the same time it yields to greater pressure and thus obviates all risk of putting the lever out of adjustment.

Later in my experiments I made the following improvement in the writing point (Fig. 1, *T'*). Instead of trimming the foil down to a point for recording on the plate, I took part of a fine sewing needle, and, after running it twice through the foil, I fastened it firmly in position by a thin film of sealing wax. By this arrangement the elasticity of the aluminium was retained, while the point of the needle insured a more delicate line than could always be gotten with the foil point.

An improved Fick myograph (see Journ. of Physiol., Vol. II, p. 164) was used for recording the curves. The time was given by a tuning fork making 200 vibrations to the second. Some of the curves were taken on smoked gelatinized paper, and the tracings set with a solution of shellac in alcohol. The other curves were taken directly on the smoked glass plate, set with turpentine, and photographed as blue prints.

It will be observed that the arrangement which I have employed necessitates the passage of the current in the longitudinal direction through the muscle. This was done intentionally, for the results of previous work by different observers, though conflicting, in general go to show that a greater contraction can be obtained from the same strength of current when passing longitudinally through the muscle than when taking any other path. Furthermore, under these conditions diffusion of the current is at a minimum.

Unless otherwise specified, the closing current is to be understood as having been used. Direct currents were used in preference to induced, for Brücke[1] has shown that curarized muscles, like muscles in certain pathological conditions, are more sensitive to the stimulus of direct than of induced currents.

The first question that I undertook to decide was, whether curarized muscle obeys Pflüger's law for the stimulation of nerves;

[1] Sitzungsberichte d. Wiener Akademie, 1867 and 1868.

that is, does the stimulus start from only one electrode, viz., the cathode on closing, and the anode on opening the current.

Of previous investigators who have taken up this question, von Bezold[1] and Engelmann[2] answer the question in the affirmative, while Aeby[3] and Bernstein[4] take the opposite view. Aeby[5] admits that the stimulus at the cathode is stronger, but denies that it arises there sooner than at the anode. Bernstein[6] asserts most positively that the muscle is stimulated at both anode and cathode, and that the stimuli are equal and simultaneous. In my experiments the muscle was essentially in the same position as in the experiments of Bernstein, and my results are of interest when taken in connection with his.

I find that *when the muscle is moderately weighted it will obey Pflüger's law, but when the weight is so small that its resistance to the contraction of the muscle is insignificant, it apparently does not obey this law, but acts as described by Bernstein.*[7]

In one experiment where the muscle was not weighted at all, one electrode was 1.5 mm. from the section of muscle on which the glass strip was laid, while the other was 70 mm. distant. No difference could be detected in the length of the respective latent periods of the contraction waves when the polarity of the electrodes was reversed, although, according to Pflüger's law for nerves, the stimulus would have to travel *nearly fifty times farther* when the cathode was at the distant electrode than when it was at the one near the recording section.

This is shown in curve 1. When this curve was taken the muscle was slightly weighted, but not enough to offer any appre-

[1] Untersuchungen über die Elektrische Erregung der Nerven und Muskeln. Leipzig, 1861.

[2] Pflüger's Archiv, Vol. III.

[3] Untersuch., &c., op. cit.

[4] Op. cit.

[5] Op. cit., p. 58.

[6] Op. cit., pp. 81, 82.

[7] This apparent difference is probably due to the fact that, when the muscle is unweighted, the stimulus produces a regular contraction which starts from the cathode but which causes the whole muscle to jerk. When the muscle is stretched by a considerable weight this is prevented, and we have recorded only the true wave of contraction as it proceeds along the fibre. Bernstein worked with the adductor of the frog's thigh and used no stretching weight.

ciable resistance to the contraction. One curve appears to have a longer latent period than the others; this is due to the fact that it is recorded slightly above the base line. If, by the eye, it be lowered to the base line, it will be seen that its latent period is about the same as that of the other two. The letters *R* and *L* denote the position of the reversing key to right or left, and consequently show opposite polarities of the electrodes. The two *R*'s are simply for control.

Curve 2 shows the effect of loading with a weight sufficient to keep the muscle well stretched. On the same base line two curves are taken, the polarity of the electrodes in one curve being the reverse of that in the other. *The curve with the shortest latent period is always obtained when the cathode is nearest the recording section of the muscle.* The difference between the latent periods of the two curves depends upon the distance between the electrodes, but it is extremely interesting to notice that the two do not vary in the same ratio, but in such a way as would make it appear that the rate of propagation diminished as the stimulus proceeded along the fibre. This point is suggestive and needs further investigation, and I hoped to publish something more definite upon it had I been able to complete the research.

While working up this subject I also repeated the experiment of von Bezold, which was repeated later by Engelmann, both of whom obtained the same result. Bezold's method was to clamp the muscle firmly enough to prevent a contraction wave from passing the clamp, but not so tightly as to destroy the conductivity of the fibres. The electrodes were so placed that the clamp came between them.

In Fig. 2 may be seen my arrangement of the apparatus for this experiment. If the muscle obeys Pflüger's law we should get a longer latent period to the curve when the cathode is at *e* than when at *e'*, for the contraction of the segment of muscle above the clamp *C* only serves to raise the weight *X*, and does not affect the recording lever; hence there would be no contraction recorded until the stimulus had traversed the length of muscle from *e* to *C*. If the stimulus starts from the anode as well as from the cathode, the latent period in each case should be the same; for the moment the segment below begins to contract, the lever will begin to write the curve.

The results of this experiment may be seen in curve 5. The sign of the curves on the plate is the same as the polarity of the electrode below the clamp when that particular curve was taken. Curve 5 consists of five curves. The numbers at the respective curves show the order in which they were taken. When the first curve (+ 1) was taken there was defective contact at one part of the circuit, and hence the curve is worthless. The trouble was removed before the other curves were taken. The two + curves and the two — curves corroborate each other. The difference between the curves is striking. The curves marked minus (—) may be taken as the normal curve of the muscle when stimulated directly on the part which records the contraction. In the curves marked plus (+) we see that not only is the latent period prolonged by the length of time it takes for the stimulus to pass from *e* to below *C*, but *the whole character of the curve is altered.*

Bernstein has pointed out that the wave of contraction is diminished in intensity as it proceeds along the fibre, and this observation is confirmed by the diminished height of the curves marked + in curve 5, as compared with those marked minus.

The curves show also another interesting point which, so far as I know, has not been mentioned before, viz., that the curve length or *duration of the contraction* is apparently *increased* as the wave travels along the fibre. This point receives additional confirmation when we examine the curves on curve 3. Here the difference in *wave length* is not so marked as in curve 5, for the electrode *e* was just above the clamp, while in the former case it was considerably above. Bearing this in mind, it is interesting to notice that there is a considerable difference in the height of the curves. This would tend to show that the resistance of the muscle within the clamp is greater than in the remaining portion. It is therefore questionable whether it is advisable to employ this method in measurements involving time, such as determining the rate of propagation of the contraction wave, etc.

Another point which I have observed in confirmation of previous observers, supporting the assumption that curarized muscle as well as nerve obeys Pflüger's law, is, that if a very much exhausted muscle be stimulated by a minimal current, a single twitch may be seen at the cathode, while no change is noticed at the anode. This may be explained by the view of Aeby, viz.,

that a stimulus also takes place at the anode but is too weak to cause a contraction.

In determining the rate of propagation of the stimulus from the contraction, the apparatus described in my first experiment was used. All the wires (seven) on the glass plate (Fig. 1, *B*) were connected with the binding screws (b^1–b^8) of the moist chamber, so that the muscle could be stimulated at seven different places without disturbing it after it had once been placed in position. In applying the stimulus the cathode was always kept nearest the recording section of the muscle, so that no complications from an electrotonus could arise. The muscle was stimulated at different known distances from the recording section, the distance in some cases being equal to zero.

In the latter case the interval between the stimulation and the contraction represents simply the latent period of the muscle, while the corresponding interval in the other cases will depend upon the distance of the cathode from the recording section. From a comparison of the intervals of the different curves the rate of propagation of the stimulus can be determined.

From the work as far as completed I can only say that the rate may be given roughly as from 2–3 meters per second. There are great variations between the muscles of different terrapins, a fact pointed out by all previous investigators. The terrapin's muscle is more sluggish than the muscles of the frog, hence the above value is not far from what we should expect when compared with the rate as found by Bernstein and Valentin for frog's muscle, viz., 3–4 meters per second.

The other results of interest obtained from my work may be briefly stated as follows:

The height of the curve depends on the strength of the stimulus, but the latent period does not. The strength of the current was varied by a resistance box, and a galvanometer was interpolated in the circuit as an index to the strength of the current employed.

When the order of polarities at a given electrode is +, −, +, −, *etc., for successive stimuli, the corresponding curves are higher than when the order is* +, +, +, etc., or −, −, −, etc. This confirms a statement made by Hermann,[1] that the passage of a constant current through a muscle increases its irritability for

[1] Hermann's Handbuch der Physiologie, Vol. I, p. 95.

the closing of currents in the opposite direction, or for the opening of currents in the same direction. Compare the curves + 1 and + 2 on curve 3. The curve + 2 is lower than + 1 because it was taken immediately after it and the current passed through the muscle in the same direction.

I have also noticed, as have previous observers, that when the current is sent into a muscle, it frequently relaxes before contracting. This is shown in curve 4, by a sinking of the curve below the base line. This phenomenon seems to have some connection with the stretching weight of the muscle, as it is not perceptible unless the muscle is well weighted; it is the more marked the heavier the weight.

From this it would appear as though the muscle was normally in a state of slight tonus which was for the moment destroyed when the stimulus was sent in, thus allowing the muscle to be more stretched by the weight.

These are the prominent results of my research, which I must now give in an uncompleted form, hoping that they may be of value to some subsequent observer who may undertake investigations in this field.

In conclusion, I would express my thanks to Professor Martin for every courtesy shown me while in his laboratory, as also for many suggestions which were of material aid to me in the prosecution of this work.

DESCRIPTION OF THE FIGURES.

FIG. 1.—Arrangement of apparatus in experiment for determining the rate of propagation of stimulus wave as given by the contraction, and also for ascertaining whether curarized muscle obeys Pflüger's law for the stimulation of nerves.

A. Moist chamber, with a large hole in the bottom, shown just below the letter *M* in the figure.

U. Upright which supports the moist chamber.

D. Heavy iron base into which the upright *U* is screwed.

b^1–b^6. Binding screws to the moist chamber. These were connected with the electrodes of the plate *B*, and with the battery as shown in Plate 3.

B and *B'*. Glass plate on which the muscle was laid. It consists of a piece of window-glass 125 cm. in length by 3 cm. in width.

Along its long edge on each side was cemented a narrow strip of glass, best shown in B'', which represents a section of B' taken at the electrode e^2.

The electrodes were platinum wires, soldered with sealing wax to the strip a and stretching across to the corresponding strip on the opposite side. They are seen at e^1, e^2.....e^7 in B', and in B'' are represented by the dotted line, which shows their position when bent over the muscle so as to make good contact both above and below.

Between the electrodes and the glass plate beneath is slipped a strip of glass, cut to the proper width and not quite as thick as the strips a and a' in B''.

This arrangement possesses a double advantage. In the first place the strip can easily be removed and thoroughly cleaned after each experiment; and secondly, we may be assured of better contact, when the strip does not come quite up to the electrodes, than if the latter were stretched directly across the flat surface of a glass plate.

p^1–p^4. Four pins, soldered to the strips a and a' with sealing wax. Their purpose is to guide the glass strip S as it is raised by the contraction of the muscle, and to keep it from slipping from its position.

S. A narrow strip of thin glass which rests on the muscle.

s–s'. Pieces of silk thread, soldered to the ends of the glass strip S with sealing wax. These run down to k, the point where they unite, and are fastened to the silk thread which suspends the pulley P.

P. A pulley over which passes a silk thread fastened at one end to L, the recording lever, while the other winds around the screw S', by means of which the lever can be quickly and accurately adjusted to any position in the vertical plane.

G. A glass rod drawn out and bent as shown in the figure. It is so placed as to keep the thread from touching any part of the lever or its adjustable attachment F.

T. Tip to the recording lever L. It consists of a piece of aluminium foil, with a needle stuck through it and fastened rigidly with sealing wax.

M–M'. The muscle in position on the plate. The thread from the end M of the muscle is passed through the hole h', and made fast to a hook not shown in the figure. The cord from the end M' passes through the hole h and suspends the stretching weight, X, of the muscle.

FIG. 2.—Description of apparatus used in repeating Bezold's experiment.

A. Moist chamber as in Fig. 1.

M, *M'*. Muscle. (In this experiment only one group of the muscles making up the retractor was used.)

H, *H*. Horizontal arms supporting a glass tube over which, as over a pulley, was passed, from the muscle, the string *J* which suspended the weight *X*.

H'. Horizontal arm supporting the clamp *C*.

C. Clamp, made from an adjustable burette clamp, and lined with sheet rubber in order that the current might not be conducted by the metal.

J. String from end of muscle to weight *X*.

X. Weight which could be raised freely by the contraction of *M'*.

J'. Piece of silk running from tendon of muscle directly to the recording lever *L*. The lift in this case was so great that the pulley was dispensed with.

L. Recording lever, made from a thin strip of light wood, about half the length of the lever in Fig. 1.

F. Foster's adjustable lever attachment as in Fig. 1.

W. Reversing key, or wippe, by which the polarity of the electrodes could be changed.

Cu. Copper; *Zn*. Zinc of battery.

e, *e'*. Electrodes on muscle. These were made of platinum wire, and made to encircle the muscle.

FIG. 3.—The electrical connections used with the apparatus in Fig. 1.

K^1. Universal connecting key, by which any one of the binding screws of the chamber *A* may be made the positive or negative pole at will.

It consists of an ebonite plate, to which are screwed seven isolated plates of brass, each having a semicircular excavation at both ends, parallel to the line of the ends of these plates. On each side of the block are screwed the brass strips *N* and *N'*, with excavations in them opposite those of the plates. These strips are not in contact with the plates, but the plates and strips may be connected by a brass plug which fits into the circular hole made by the excavation in each. Each of the plates is connected with one of the electrodes of the moist chamber. This connection was made by wires insulated with silk of different colors, so as to avoid confusion. The brass strip *N* was connected with *R'* directly; the strip *N'* was connected with *R* by the path *N'*, K^1, K^2, *R*.

R, *R'*. Two binding posts connected by a tongue of brass which is knocked away by the myograph in its swing. The posts *R* and *R'* are connected with the poles of the battery, so that when the tongue is up, and *R* and *R'* connected as represented by the dotted line, we have the primary circuit: *Cu*, K^3, *W*, *R'*, *R*, *W*, *Zn*. Into this circuit is inserted a Du Bois Reymond key, K^3, for convenience in breaking the circuit, and also a reversing key, *W*, by which the direction of the current may be changed.

If *R* and *R'* had no connections except with the battery, it is clear that when the circuit is broken between them they become the positive and negative poles respectively. Since *R* and *R'* are connected with the strips *N'* and *N* respectively, it follows that these must become the positive and negative poles when the connection is broken between *R* and *R'*. In the same way, by connecting the strip *N* or *N'* with one of the brass plates between them, we confer its polarity to that plate, and thence to the binding screw of the moist chamber with which the plate is connected, and thence, in turn, to the electrode on the muscle. As any one of the brass plates may be connected with either of the strips *N* or *N'* at will, it follows that we may make any one of the electrodes the cathode or the anode at pleasure.

When everything is ready to record a curve, the tongue between *R* and *R'* is in position, closing the circuit *Cu*, K^3, *W*, *R'*, *R*, *W*, *Zn*. As the myograph swings the tongue is knocked away, the connection between *R'* and *R* is broken, and the current takes the longer circuit *Cu*, K^3, *W*, *R'*, *N*, through the plug to one of the brass strips, thence to the moist chamber, returning through another wire to its corresponding plate, thence through a plug to *N'*, K^1, K^2, *R*, *W*, *Zn*.

K^1. A Du Bois Reymond key for opening or closing the secondary circuit.

K^2. Key for throwing the galvanometer *G* in or out of the circuit.

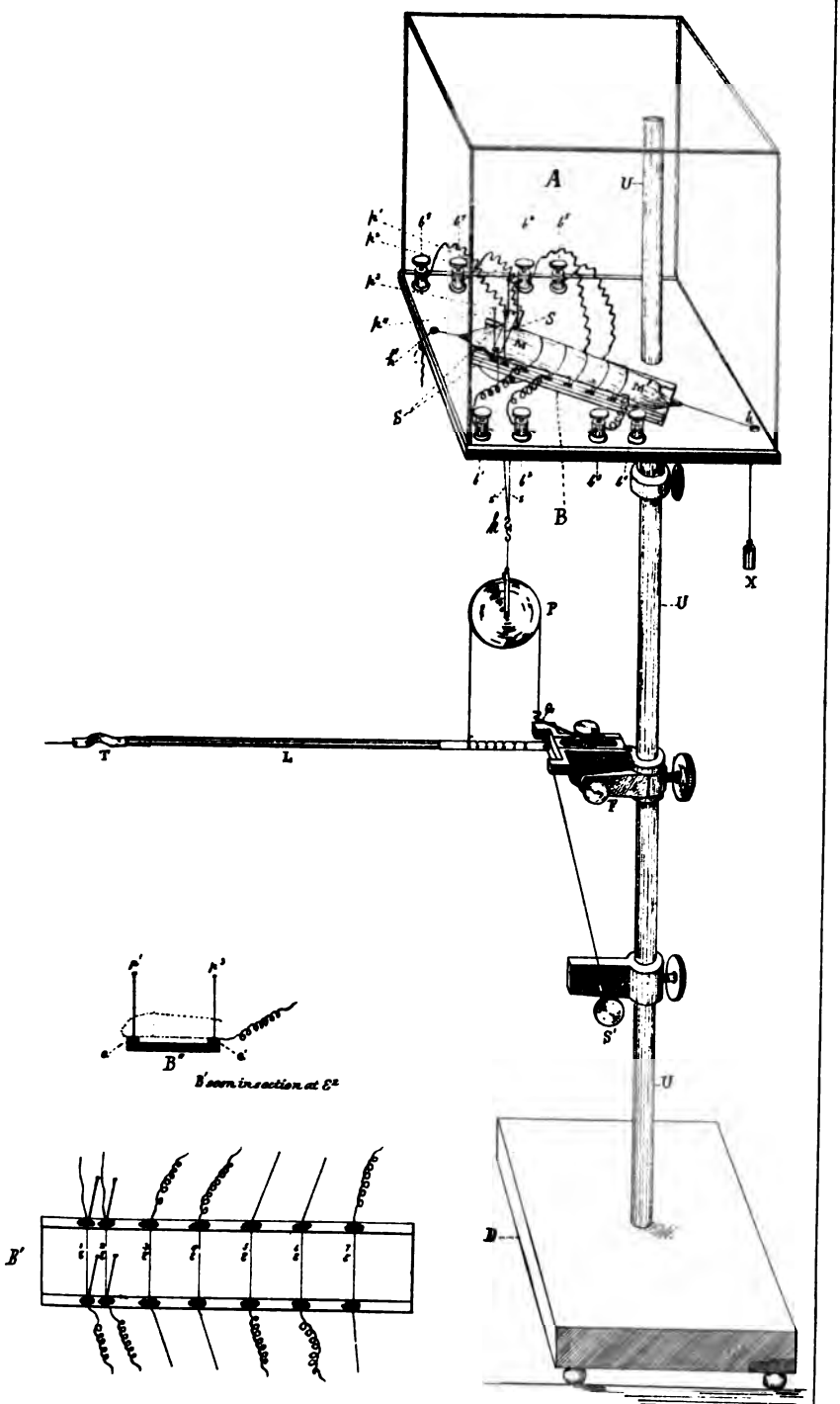


Fig. 1

PHOTO LITH BY A HOEN & CO BALTIMORE, MD.

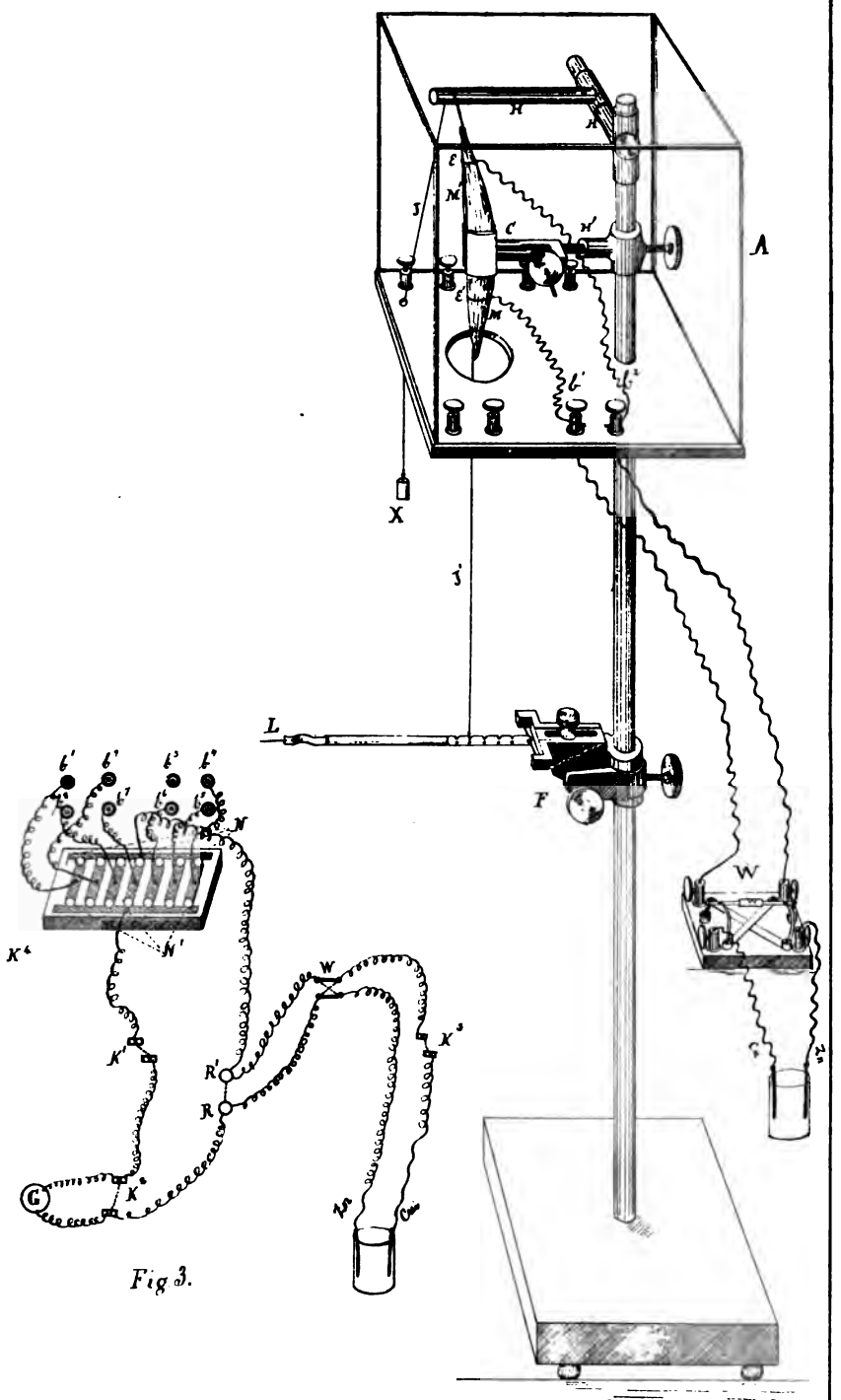


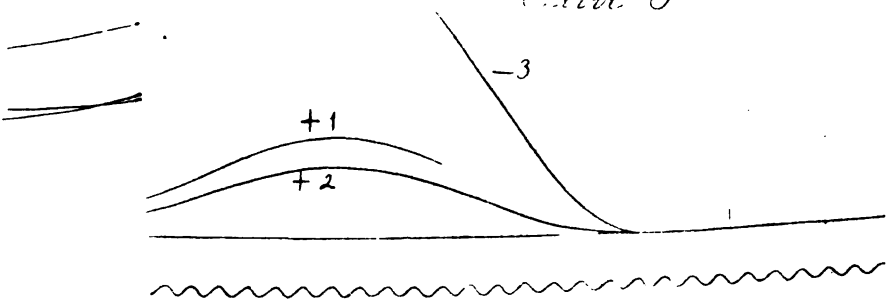
Fig 3.

Fig 2.

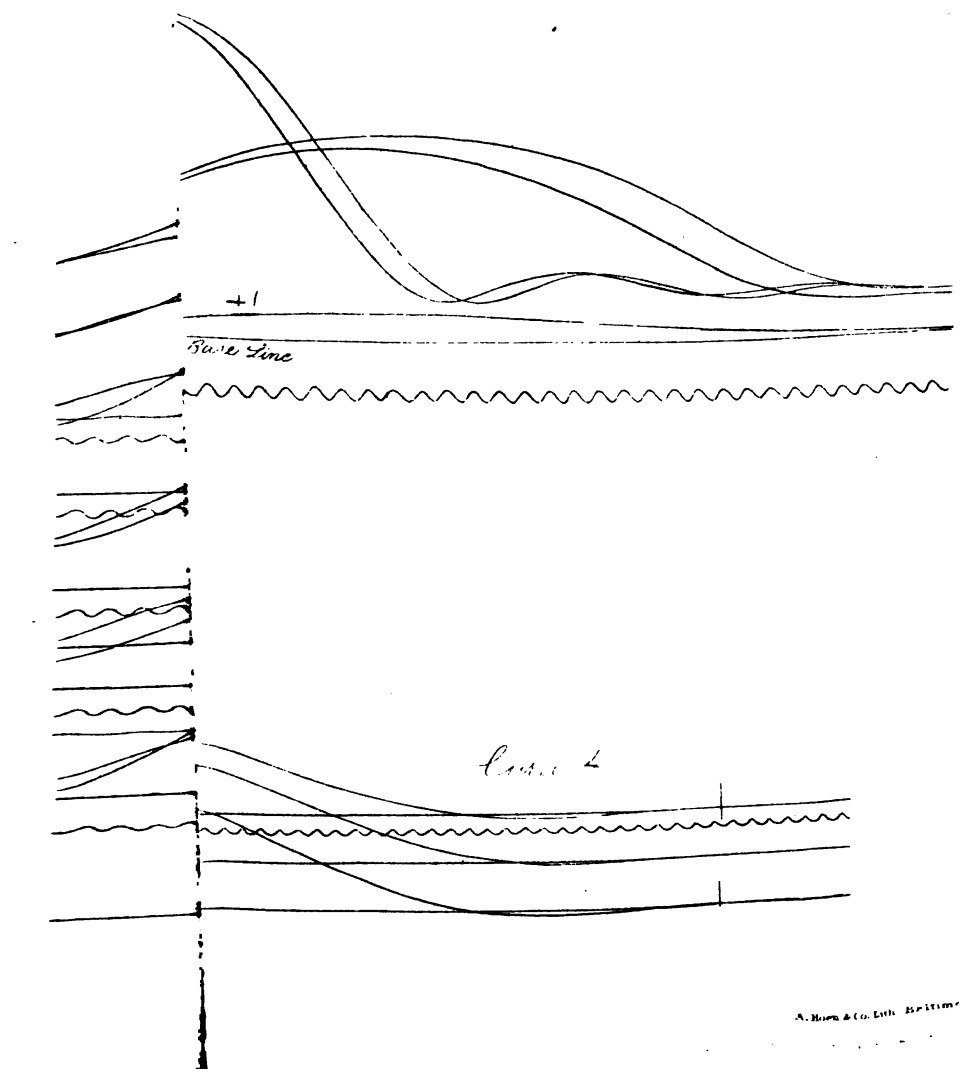
PHOTO LITH BY A. HOEN & CO. BALTIMORE MD

STUDIE

Curve 3



Curve 1



Curve 2

A. Hoen & Co. Lith. Baltimore

EXPERIMENTS ON TETANUS AND THE VELOCITY OF THE CONTRACTION WAVE IN STRIATED MUSCLE.

By JOHN P. CAMPBELL, A. B., late Fellow of the Johns Hopkins University. With Plates X, XI, and XII.

The neck retractor muscles of the terrapin are peculiarly well adapted for the study of the phenomena of muscle contraction. With the exception of the one disadvantage that a muscle-nerve preparation cannot be made, they possess every other valuable feature found in frog muscles, and have at the same time larger size and greater vitality.

The retractors of the neck consist of a group of four distinct muscles, two on either side of the middle line. Posteriorly they are attached very close together to the pelvic arch, and their attachment may run up the sides of the spinal column for a variable distance. Anteriorly the shorter muscles are inserted into the transverse processes of the cervical vertebræ, while the longer muscles pass on and are inserted into the ventral surface of the palatine bones by short tendons which sometimes fuse with one another.

The long muscles are especially noteworthy. Their length when extended is approximately equal to the length of the carapace of the animal, and in general appearance they resemble the sartorius of the frog. The fibres run perfectly straight. At the posterior end they are inserted directly into the sides of the sacral vertebræ, while the tendon at the anterior end is exceedingly short and does not extend up into the substance of the muscle at all. Furthermore, the cross area of the muscle is very uniform throughout its whole length.

In spite of this combination of valuable qualities these muscles have been little investigated, owing no doubt to the fact that Chelonia do not occur in many parts of Europe. During the winter months it is easy to obtain in Baltimore specimens of the "diamond back" terrapin (*Malacoclemmys Palustris*) from four

to six inches in length, and at Prof. Martin's suggestion I have undertaken the study of two of the problems, hitherto unsolved, which the muscles of this species present. First, to determine the minimal number of stimuli per second necessary to tetanize them; and second, to determine the rate at which the wave of contraction propagates itself along the muscle.

I desire here to acknowledge my indebtedness to Prof. Martin for the facilities placed at my disposal, for his kindness in having apparatus constructed for me, and for advice and suggestions; also to Dr. Howell for valuable suggestions and criticisms given during the course of my work.

Part I.—Experiments on Tetanus.

Description of Apparatus.

Induction shocks were used for stimulating in all of the experiments. Several methods for interrupting the primary circuit were employed. In some of the earlier experiments Kronecker's capillary contact interruptor[1] was used, but my experience with this was not very satisfactory. In the first place its motion was very irregular at slow rates of vibration, and, besides, the fact that opening and closing shocks alternate is a rather grave objection. The interval, too, between any closing shock and the succeeding opening one is not perfectly constant, but irregularities sometimes occur, owing to the mercury being drawn out by the wire, thus slightly delaying the moment of breaking the circuit. In addition, there is no way to control the rate of vibration so as to set it in advance to interrupt at any desired rate, and the only way in which the rate can be calculated is from the tracings gotten from the muscle itself. The apparatus often occasions annoying delays, and requires too much attention to be used where anything else is to be observed.

In some of the following experiments I employed Ludwig's interrupting apparatus.[2] This had one great advantage over that of Kronecker in giving only one kind of shock, *i. e.* either opening or closing as desired. In using this I endeavored to

[1] Verhandlungen der Physiologischen Gesellschaft zu Berlin, 1877, No. 3.

[2] See article by Lukjanow in Arch. für Anat. und Phys. Phys. Abt. Supplement, 1886, p. 130.

calibrate it in advance by putting a chronograph in the circuit and determining the number of interruptions per second for any given weight and adjustment. This was not very satisfactory, because of the difficulty in keeping the rate constant under the same circumstances. The manner of making and breaking the contact is at least open to suspicion also. The pins on the cylinder allow the movable arms of the keys to spring back with great suddenness. At rapid rates, then, it may be questioned whether the contact is made at once. A rebound might easily occur in either key, and the result would be that the muscle would receive more stimuli than was supposed, and that both kinds of shock would get through to the muscle by reason of the short circuit key being open when the shock not desired was produced.

This was then given up in favor of an interruptor which was constructed for me in this laboratory. (See Plate X.) In part it is a modification of Ludwig's form. The axle, supported upon a rubber base, carries two eccentrics which may be adjusted in any way desired. The rims are grooved, and in these grooves lie two steel rods which constitute the upper part of the contact keys. These rods are kept pressed firmly against the eccentrics by springs, but the adjusting screws below are so regulated that at the moment of contact the rod very slightly leaves the eccentric, thus making the contact more certain.

The advantage of the eccentric over the pins used in Ludwig's apparatus is obvious. Contact is not made by allowing the movable arm of the key to spring suddenly from its most distant point against the fixed arm, but the motion of the eccentric brings the two together as slowly as the rate of rotation of the axle will allow, thus diminishing greatly the danger of a rebound.

Key *a* needs no special mention. Key *b* is so constructed as to interrupt two entirely distinct circuits simultaneously. The structure of this key is made plain by the diagram (Plate XI, Fig. 1) representing the front and side views. The central part of the vibrating arm is made of rubber, and on either side is fastened a thin plate of brass, bent out at right angles below, and covered with platinum on one face to form the contact with the screw from the fixed arm. In this way the two circuits are kept entirely distinct, and wires are led off from each side, as

shown in the figure, and connected with the binding screws. In order to keep the surfaces bright, a vessel containing kerosene is placed under the two keys, and may be raised and lowered as desired.

One side of the double key is used to interrupt the primary circuit, and the single key *a* is placed in short circuit with the secondary coil. If now the eccentrics are so placed that their long axes make an angle of 90 degrees with each other, one shock will result from each rotation of the axle. If it is so arranged that the short circuit key opens first and, after the axle has turned through 90 degrees, the primary circuit is opened, then the opening shock will get through to the muscle. But the short circuit key also closes before the primary circuit, hence the closing shock does not reach the muscle. This arrangement was used in all of my experiments.

For rotating rapidly a water motor was employed. This was connected with a closed mercury manometer provided with a scale, and in this way, knowing the water pressure necessary to give any desired rate, it was easy to set the machine going at that rate in advance. Finer adjustments could be made by means of a spring pressing on the hub of the fly-wheel, but in practice this was rarely used, since the motor carried pulleys of several different sizes, and by getting a combination which would give nearly the desired rate, slight changes in water pressure would do all that was desired.

The motion of the apparatus is very regular. Slight and often sudden variations in pressure frequently take place in the city supply, and of course a tank at a high elevation would have been a more nearly ideal arrangement, but I found no great difficulty in keeping the rate constant for any length of time. However, supposing that any irregularity of motion should occur, the side of the double key hitherto unaccounted for gives us a means of detecting it. This is placed in the circuit going to a chronograph which writes upon the drum immediatety under the muscle tracing. If then a series of opening shocks is being used, every ascent of the chronograph line from its lowest position (current closed) to its highest position (current open) indicates the moment of opening of the primary circuit and stimulation of the muscle.

If in addition another chronograph beating at a known rate

be made to write on the drum, we have entire control of the number of stimuli which the muscle receives. For this purpose an electric signal was used, the circuit being interrupted by a tuning fork making 50 double vibrations per second.

Every record then consisted of three lines. The upper was the tracing made by the muscle; the middle showed intervals of $\frac{1}{50}$th sec., and the lower gave the number of stimuli which the muscle received.

To make periods of rest and activity alternate at regular intervals, an ordinary drum kymograph was used. Wires were soldered to the drum, and these dipped down into a mercury trough as the drum rotated. So long as the wires were in the mercury, the current would entirely short circuit through the drum. By bending any number of wires so that they would clear the surface of the mercury, the current would get through to the muscle for constant periods and at regular intervals.

In some of the experiments when the muscle was to be kept at the room temperature, an ordinary glass moist chamber was used. In others this was replaced by a double walled metal cylinder, through which water of any desired temperature could be passed.

The electrodes consisted of platinum wires which were looped around the upper end of the muscle about 10 mm. apart. To secure firmness these were fastened to a rubber support connected with the rubber top of the cylinder. (See Plate XI, Fig. 3.)

The amount of shortening of so long a muscle in tetanus, is so great that a lever cannot be employed unless its length is inconveniently great. Instead of this I used a recorder of the form shown in Plate XI, Fig. 2. It consists of a short piece of glass tubing, nearly sealed at both ends, and serving as a guide for a wire which moves in it freely but without having much play. To this wire is attached at the upper end a bit of cork carrying a straw with an aluminium point. The point was kept pressed against the drum by means of a weighted horse hair. With this recorder the amount of shortening of the muscle is not magnified, but faithfully reproduced.

In order to reduce the "throw" of the recorder (wurfhöhe) as far as possible, the weight was not suspended directly from the muscle, except in some of the earlier experiments, but from a

pulley some distance off. This pulley was connected with a lever, which in turn was connected with the recording arm by a wire. The actual distance through which the weight moves is so small that its full effect is exerted during the whole of the contraction. If a sudden upward blow be given to the lower end of the wire, it will not leave the finger unless the motion be very rapid. Another test showed its sensitiveness even better. A tuning fork beating 50 per second was set vibrating by an electric current, and the lower end of the wire was placed upon one of the arms. The tracing made upon the drum faithfully represented the number of vibrations of the fork.

This apparatus then is capable of showing the slightest changes in the position of the lower end of the muscle, within at least 50 vibrations per second.

The connection from the muscle to the recorder must not be made by a thread, but by something more rigid. In my experiments a fine platinum wire was used.

The precaution must also be taken not to place the recorder on the same upright that carries the two chronographs, since their motion makes the muscle curve irregular.

Preparation of the Muscle.

The terrapins were always placed in a warm room for several hours preceding an experiment, the advantage of this being that a smaller amount of curare sufficed to put the animal thoroughly under its influence, and it also had its effect more quickly; three or four minims were always sufficient to thoroughly curarize a small terrapin, and usually in ten minutes or less. The curare was given by trephining a small hole in the plastron directly over the heart, and inserting the needle of a fine hypodermic syringe into the apex of the ventricle. After the curare had taken effect, the animal was pithed, the bridges of the carapace were sawed through, and the skin cut away from both carapace and plastron posteriorly. Both ilia were then cut through with bone forceps, and the shoulder girdle was broken away from the carapace. The skin of the neck was then cut on the middle line ventrally, and the trachea and esophagus removed, the hyoid bone being cut away on both sides. If now the hind legs are drawn forward, all the viscera will be carried with them, leaving

behind only the group of neck retractor muscles. The anterior end may be easily dissected away from its insertion. The posterior end presents more difficulty, but by using a sharp chisel, with not too wide a blade, the vertebræ may be separated from the carapace with little or no injury to the muscle, and without consuming much time. The two sides may be separated with scissors without injury to the muscle, by cutting up the spinal canal.

On the Minimal Number of Stimuli Necessary to Produce Tetanus.

Marey[1] found that a muscle from a tortoise was held in almost perfect tetanus by three stimuli per second. No statements are made as to what temperature was employed, and indeed it is not stated what muscle he used, or what the species of the animal was. The muscle was enclosed in a *pince myographique*, thus recording changes in thickness and not length. Whether the muscle carried any weight is also not stated.

My first object was to see how nearly the curarized neck retractors of the terrapin corresponded with this, and also to study the effect of changes of temperature and strength of stimulus.

I made in all sixteen experiments upon this subject, and the results were very constant for given conditions. The figures which I give are the mean results of all my experiments. In some cases the muscle was first cooled down and the temperature then allowed to rise. In other cases the reverse order was followed, to eliminate as far as possible the effects of fatigue. For the sake of greater clearness I give the details of one experiment in which the former order was followed.

Experiment 15, *February* 14, 1888.

A terrapin was placed in a warm room for several hours before the experiment; four minims of curare sufficed to thoroughly curarize in a few minutes. The muscle was then removed in the warm room and brought into a room the temperature of which was 4° C. The weight was 75 grams suspended from the pulley, making the load lifted by the muscle slightly more than 8 grams. The stimuli were maximal from an induction coil and one carbon-bichromate cell.

[1] Mouvement dans les Fonctions de la Vie, p. 383.

After waiting five minutes for the muscle to assume the temperature of the room, and washing it with normal salt solution at that temperature, the first tracings were taken. Tetanus was produced when the muscle received one stimulus per second. (See Plate XII, Fig. 1.) Raising the temperature by passing water through the cylinder, the mean results gotten were as follows:

Temperature.	Number to Tetanize.
4	1
9	5
21	25
28	34 (See Plate XII, Fig. 4.)

These results are very constant, and variations of more than one or two per second are not met with, this slight variation being easily explained by difference in irritability owing to fatigue. As my object was not to study the effect of high temperatures upon tetanus, I did not carry this any farther. Suffice it to say that at 36–38 degrees the muscle becomes less irritable, the height of the curve is diminished, and a smaller number of stimuli per second will give tetanus. If the temperature be again lowered the irritability may be increased, though it does not return to what it was originally, as shown by the fact that a smaller number of stimuli will still tetanize.

These figures are higher than one would expect to find for an animal whose motions are as sluggish as those of the terrapin, though it should be stated that the animals with which I worked were often quite active as compared with other forms, the "slider" terrapin for example. Their activity, however, is not nearly so great as that of the frog, and yet according to Marey's determinations, so far as I can apply them to the conditions under which I worked, a greater number of stimuli is necessary to tetanize the muscles of the terrapin than of the frog.

On comparing the figures just given with those gotten in some of the earlier experiments, striking differences are found, and the cause of the differences is significant. At 22°, 14 stimuli were sufficient to tetanize, and at 31°, 16 were necessary. This was due to a difference in the recorder and its connection with the muscle. The recorder was simpler than the one represented in Plate XI, Fig. 2, and consisted merely of the glass

tube and wire, the weight being suspended directly from the wire. The connection with the muscle was by a silk thread.

For purposes of comparison I made a few experiments with frog muscles which gave rather surprising results. After curarizing, the gastrocnemius was taken out and connected by a wire with the recorder, the weight being about 8 grams. At a temperature of 25½°, 37 *stimuli per second are not sufficient to give uniform tetanus, instead of* 27, *which is the highest figure hitherto given.*[1] (See Plate XII, Fig. 3.) The sartorius requires a somewhat smaller number, being nearly tetanized under the same conditions by 35 per second. (See Plate XII, Fig. 2.)

From the foregoing it follows that we can graphically analyze a tetanus much farther than has been supposed, if the recording apparatus is sufficiently sensitive, and indeed the whole matter seems to be largely a question of sensitiveness of the recording apparatus, and consequently has little real significance. If the mere fact that a silk thread connects the muscle with the recorder makes a difference of more than ten per second in the number of stimuli necessary to produce a tetanus curve that shall not show any undulations, it follows very naturally that since the extensibility of muscle is so much greater than that of the thread, vibrations may really exist when the muscle is rapidly stimulated which do not affect the lower end of the muscle, but are absorbed in the substance of the muscle itself. If then we had a recording apparatus capable of registering very rapid alterations in length, and yet so light as not to interfere with the free movements of the muscle, and if at the same time we had a muscle which was sufficiently rigid not to fuse together the single contractions, we would expect that the muscle would respond to any rate of stimulation, and that the tracing would reproduce exactly the number of stimuli which the muscle receives. It is to be noted that in the tracings given, especially those from frog muscles, the curve does not cease to show oscillations even after tetanus has been continued for many seconds. That this is not due to unsteadiness of the apparatus is shown by the fact that the line made by the recorder is perfectly regular as soon as the stimulation ceases, although the chronographs are still vibrating. Marey[2] has shown the importance of the mechanical properties

[1] Hermann's Handbuch, Vol. I, p. 42. [2] Mouvement, etc., p. 197.

of a muscle in determining the form of a single contraction curve. The foregoing results, however, seem to justify one more step, and allow the general statement to be made that within certain limits *the more extensible a muscle is, the fewer stimuli per second will suffice to tetanize it.*

This relation is to some extent borne out by older determinations made by Marey.[1] He found that the hyoglossus of the frog is tetanized by 10 stimuli per second, while the gastrocnemius requires 27. It has been stated that there is a difference of 2 per second between the gastrocnemius and sartorius of the frog. The difference is really greater than this, since the curve of the sartorius is more nearly a perfect tetanus than that of the gastrocnemius. The sartorius in fact almost agrees with the terrapin muscle, which it so strikingly resembles in mechanical properties. The greater rigidity of the gastrocnemius, then, is the explanation of the fact that changes which take place in the upper part of the muscle are more faithfully communicated to the lower part, and hence are seen in the graphic record.

Effect of Strength of Stimulus.

Kronecker[2] states that when very weak stimuli are used tetanus may be produced, but with his interruptor irregularities often appear in the curves. These he supposes to be due to inequality of contact owing to the mercury being drawn out by the vibrating wire.

In order to see if any irregularities would be found when the contact was perfectly reliable, and especially when the inequality of opening and closing shocks could not influence the results, I made a few experiments on this point.

In every case I found the curves to be perfectly regular, but the rise was often exceedingly slow. (See Plate XII, Fig. 6.) Tetanus may be produced by stimuli which singly are without effect, and which even when repeated at intervals of one second do not cause any contraction.

These experiments were made early in the course of my work, and before the recorder had reached its final form. The figures gotten are not, then, strictly comparable with those for maximal stimuli, but on comparing them with the earlier results

[1] l. c. p. 382. [2] l. c.

for maximal stimuli, very little difference is observed unless the stimuli are very weak. When the stimuli are just minimal, a smaller number will suffice to tetanize, in certain cases 8 per second being sufficient under the conditions which would require 12–14 stimuli if maximal or nearly so. (See Plate XII, Fig. 5.)

This becomes plain when we consider the single contractions, which are very much prolonged and not by any means comparable with the upper portion of a maximal contraction curve gotten under the same conditions. This is in accord with the general condition to which Sewall[1] has called attention. He found that in summated contractions the higher the contraction the more sudden was the fall. He explained this by supposing that at favorable intervals of summation "the contractile energy of the muscle is condensed and used up rapidly in doing work, leaving the muscle an inert body to be rapidly stretched as such by the suspended weight, when the shortening phase of its contraction is over, while in a single contraction the contracting energy is not expended at the greatest shortening, but resists for a time the fall of the appended load."

The behavior of weak single stimuli is probably to be explained in the same way. Not all of the energy liberated by the stimulus is used up in the ascending phase of the contraction, but a certain amount is used after the muscle has reached its greatest height. The effect of this is to hold the muscle at its position of greatest shortening longer than when the stimuli are stronger.

Another point which was very constantly observed may be worth mentioning. When stimuli of moderate strength, considerably less than maximal, were sent in to the muscle at a rate of 6–8 per second, they very soon fuse into a perfect tetanus, *i. e.* after a much smaller number of stimulations than would be necessary to produce fusion if the stimuli were maximal. This is probably owing to fatigue working through the changes which it produces in the mechanical properties of the muscle, and is in accord with the observation of Kronecker that when weak stimuli are used the fatigue curve falls more suddenly than for strong stimuli.

[1] On the effect of two succeeding stimuli upon muscular contraction, *Journal of Physiology*, Vol. II, p. 175.

Part II.—On the Rate of Propagation of the Contraction Wave.

For determining the rate at which the contraction wave propagates itself along a muscle, a variety of methods have been employed. Aeby,[1] who was the first worker in this field, used the gracilis of the frog. This was placed on a horizontal surface, and two levers which wrote on a myograph were allowed to rest upon different parts of the muscle as far removed from each other as possible. If then the muscle was stimulated at one end, the lever nearest the point of stimulation would rise first, and from the difference in time and the length of muscle between the levers the rate could be deduced. This rate he found to be somewhat more than one meter per second when the muscle was perfectly fresh, but it soon diminished as the muscle became fatigued.

von Bezold[2] and Engelmann[3] used a different method. They clamped the muscle some distance below the suspended end firmly enough to prevent the upper part of the muscle contracting, and connected wires with each end. On passing a constant current they found that the latent period differed according to the direction of the current. If, for example, they used breaking contractions, the latent period was greater when the positive pole was at the upper end of the muscle. From this they concluded that the difference must be taken up by the wave traversing the part of the muscle which is prevented by the clamp from contracting. In this way the rate was found to be about 1.7 meters per second.

Valentin[4] used the same method as Aeby, but kept the muscle in the body. According to him, the manipulation involved in removing the muscle from the body injures it and makes the rate slower than it should be. He found the rate to be 4.28 meters per second. He got the same result as Aeby when he worked under the same conditions.

[1] Untersuchungen über die Fortpflanzungsgeschwindigkeit der Reizung in der quergestreiften Muskelfaser. Braunschweig, 1862.

[2] Untersuchungen über die electrische Erregung der Nerven und Muskeln. (Quoted in Hermann's Handbuch, Vol. I, p. 53.)

[3] Ueber Reizung der Muskelfaser durch den constanten Strom, *Jenaische Zeitschrift*, Vol. 4, pp. 302–306.

[4] Untersuchungen über Pfeilgifte, *Pfluger's Arch.* Vol. 4, p. 117.

Bernstein[1] used one lever which wrote upon a myograph cylinder. This rested upon one end of the muscle. By stimulating the other end, knowing the distance from the electrodes to the lever and the time of stimulation, the rate can be calculated. His results vary between 3.226 and 4.385 meters.

Hermann[2] used the method of electrical time measurement. The muscle (sartorius) was so placed that the lever of a Helmholtz "frog-interruptor" rested on one end, and the stimulus was sent in at the other. If the time-measuring current be passed through a ballistic galvanometer at the moment of stimulation by means of a double key, and continue to pass through until it is taken out by the thickening of the muscle breaking the contact, the deflection of the needle will give the time which the wave occupies in traversing the given length of muscle. With this method he obtained results varying from 1.667 to 3.313 meters per second. One determination made on the muscle of a tortoise (*Testudo graeca*) gave the rate 1.829 meters per second. None of these methods are entirely free from objection, and much of the work is also open to criticism.

The graphic method of recording is not satisfactory. The actual amount of thickening is so small that the lever must be very long, and this brings in two objectionable features: 1st, the weight of the lever, and 2d, the slowness of rise, making it very difficult to locate accurately the point at which the ascent from the base line begins. Aeby's work is also rendered less valuable by the fact that he used the gracilis of the frog, which, as Hermann pointed out, is rendered unfit for this purpose because of its having a tendon which interrupts the continuity of the muscle substance.

The method used by von Bezold and Engelmann is open to the objection that clamping injures the muscle. This is shown by the very rapid decrease in rate which their figures give, and Engelmann, at least, attributes this to injury to the muscle. This method would give the velocity of the wave of stimulation (reizwelle) rather than the wave of contraction (contractions-

[1] Untersuchungen über den Erregungsvorgange im Nerven- und Muskelsysteme. Heidelberg, 1871, pp. 76–94.

[2] Neue Messungen über die Fortpflanzungsgeschwindigkeit der Erregung im Muskel. *Pflüger's Arch.* X, pp. 48–55.

welle), although the two are probably identical. It appears somewhat strange, however, to determine the velocity of the contraction wave in a portion of the muscle which is not allowed to contract. The clamp, too, would act as a heavy weight which the muscle was striving to lift, and a much more serious objection to the method will be pointed out later in considering the effect of the load on the rate of the wave. Bernstein's work may be objected to on the ground that the conditions under which he measured the length of muscle traversed by the wave do not correspond to the conditions under which the muscle contracted. In contracting it carried no weight. Every part was free to move except the part immediately connected with the lever. The muscle then was simply stretched out and allowed to assume its own position. Instead of measuring it in this condition and assuming that the distance from the electrodes to the lever represented the length of muscle traversed by the wave, he stuck needles through the muscle at these two points and then stretched it by a weight of 30 grams. The muscle then contracted with no load, but was measured with a considerable load. This fact explains in a measure Bernstein's high results.

Hermann's method is without question the most sensitive of all those mentioned. The advantage over any method in which the actual thickening of the muscle is recorded graphically, lies in the fact that the very beginning of the process of contraction at the spot under the lever is accurately determined. The lever can be so accurately adjusted that it requires almost no force to lift it, and the total mass of the lever may be much less than in the rather cumbersome levers used by Aeby, the inertia of which must have been very considerable.

There are certain disadvantages also connected with this method. In the first place it is a difficult method to use. In order that the time may be deduced from the amount of deflection of the needle, it is necessary to calibrate the galvanometer in advance by allowing the time-measuring current to pass through it for known periods. This is troublesome and tedious, moreover must be repeated for every experiment. So much time also is required for each observation that but few can be made on the muscle while it is in its freshest condition.

It should also be borne in mind that while the ballistic galva-

nometer is a very accurate measure for minute fractions of a second, it is not so reliable when the periods are somewhat longer. The essential condition is that the needle must be so heavily loaded that it will not begin to move until the whole current has passed through, and the longer the period the more difficult it is to be sure of this condition being fulfilled.

Hermann also measured the muscle while weighted, but had no weight when the muscle contracted. This makes his results somewhat high, since the wave really traverses more of the muscle substance than was measured.

Description of Apparatus.

My endeavor was to construct an apparatus which would combine as many as possible of the desirable features of the methods previously used, and at the same time be as free as possible from their defects. The result finally reached is represented in Plate XI, Fig. 4. The cross-shaped base is made of hard rubber. The muscle lies upon the long arm, while a lever crosses it near one end. This lever carries a small upright arm (*b*) made of rubber and rounded at the lower end. This rests upon the muscle and may be adjusted in any direction. At this point two rubber jaws are so arranged as to slightly compress the muscle laterally. (For the sake of simplicity only one, *c*, is represented in the figure.) At this point then, the muscle is completely enclosed, three sides being fixed and the fourth movable. Any change in the cross area of the muscle, then, will raise the lever, and if the lever be carefully adjusted so that it rests very lightly upon the muscle, the slightest change will cause it to rise. The free end of the lever carries two screws with platinum points, one dipping into a mercury cup, and the other resting on a solid platinum contact, as in the Helmholtz "frog-interruptor." Wires serving as electrodes are placed at various distances along the base, one pair being immediately under the lever.

So far the apparatus is not essentially different from that of Hermann. Instead of using it to interrupt the circuit to a ballistic galvanometer, the wires were carried to an electric signal which wrote on the smoked plate of a pendulum myograph. This signal was constructed by removing the siphon from a Martin chronograph and inserting a long fine-pointed metal arm in its

place. The coils were made of coarse wire, and when supplied by one large carbon-bichromate cell, the cores become magnetized sufficiently to pull the armature down in spite of very considerable resistance on the part of the spring. When the cores are demagnetized the lever flies back with great suddenness, making the point of divergence from the base line very sharp and definite. By arranging the signal so that the lever is drawn *downwards* by the spring, the point of divergence becomes even sharper, since the base line is convex, and not straight as in a drum or spring myograph.

There is one error owing to the fact that the signal has a latent period of its own—the time required for the cores to become demagnetized. Since the determination is a difference and the error is constant, it need not of course be considered. We have then a method which is easy to use, and at the same time capable of recording very sharply the exact moment when the contraction wave first reaches the recording point on the muscle. It may be modified to measure the contraction wave as it passes from one point on the muscle to another. I made a few experiments with a double apparatus of this sort, in which two levers rested on the muscle and two signals recorded, one above the other. This gave results more directly comparable with those of Aeby, but I abandoned it because of the greater simplicity of the single lever and greater ease of adjusting. In order that the results may have any value, the two levers must be balanced in exactly the same way and press with exactly the same force upon the muscle, and it is difficult to be sure that this condition is always fulfilled.

Details of Experiment.

The two long muscles were removed together as soon as the curare had taken effect, and as quickly as possible placed on the apparatus. A string was fastened around the bone at the posterior end, and around both tendons at the anterior end. These strings were carried over pulleys and 5-gram weights attached to each in most cases. Sometimes one end was fastened and the other end free, but the results are not affected by this. The lever is next to be adjusted. The upright arm is lowered upon the muscle, and the two jaws are brought together so as to very slightly compress the muscle, but they must not touch the lever

arm. By means of the counterpoise the lever is nearly balanced. It should not press too lightly upon the muscle, for then it is harder to keep the contact preserved until the contraction of the muscle breaks it; and besides, unless the lever presses with some degree of firmness upon the muscle the very beginning of the contraction is not recorded. Experience is the best guide in this respect. The lever must press upon the muscle with sufficient firmness to be raised at the instant the muscle begins to thicken. Within certain limits this is immaterial, since the velocity is deduced from the difference between two points, and whatever error may exist is constant for both.[1]

The signal is then adjusted to the myograph so as to press lightly. The point of stimulation is determined by holding the myograph pendulum against the key in its closed position and demagnetizing the signal by raising the lever with the hand. The current is again closed, the base line marked on the plate, and the pendulum is then raised. The wires from the secondary coil are then connected with the electrodes immediately under the lever, and the pendulum is allowed to fall. The tracing thus obtained gives the latent period of the muscle, the latent period of the signal, and the time which the muscle may consume in gathering up sufficient energy to move the lever. The pendulum is again raised, the wires connected with a distant pair of electrodes, and a second tracing taken on the same base line. Into this curve enter all the factors of the first, the additional one being the length of muscle from the electrode to the lever. Any errors that may exist then are constant for both curves; hence, knowing the length of muscle and the time which the distance between the two curves measured on the base line represents, the velocity of the wave may be deduced. The time is measured by the beats of a Koenig tuning fork making 200 double vibrations per second. A fine point connected with one of the arms wrote

[1] The latent period observed is much greater than that found for frog muscle by Tiegerstedt (Ueber die Latenzdauer der Muskelzuckung: Arch. für Anat. und Phys. 1885, Phys. Abt. Supplement, pp. 113–264). It is also greater than that found for terrapin muscle when suspended instead of lying flat. This is owing to the fact that the suspended muscle may be much more accurately adjusted to the lever than the muscle lying flat. The muscle can be made more tense without breaking the connection at the end of the lever. The shorter period, then, is due simply to better mechanical conditions.

directly upon the plate of the myograph. The velocity of the plate was quite rapid, the pendulum being raised to a considerable height, consequently the beats are considerably drawn out, making it perfectly easy to measure intervals of .0005 sec.

The greatest source of error is the difficulty of measuring the true length of the muscle. This has been done in different ways by different investigators. Aeby, taking no account of the weight stretching the muscle, assumed that the distance between the levers represented the length of muscle traversed by the wave.

Bernstein and Hermann both measured the muscle under different conditions from those under which it contracted, the relations being such as to make the results higher than if the same conditions had been observed in both cases.

In my experiments, as already stated, the muscles were usually loaded with 5 grams at each end. This weight stretched the muscle to nearly its length in the body when the neck was fully extended, consequently the muscle may be regarded as having nearly its normal length under these circumstances. This being the case, the distance from the electrodes to the lever represents the length of muscle traversed. The point on the muscle where the lever rests is the one fixed point, and when the muscle is stimulated both ends approach this point, to be drawn back again by the weights to their original position when the contraction is over. When one end of the muscle is fixed and the stimulus starts from the free end, there is displacement of the muscle under the lever. This is hardly an objection where it is only desired to determine the beginning of the contraction wave and not its form, since there is no displacement until after the wave has begun to pass by the lever. There is, however, more danger of interrupting the signal circuit before the wave actually reaches the lever, when one end is fastened, than when both ends are free, consequently the latter arrangement was the one usually employed.

Another point which must be carefully looked after is the strength of the stimuli. Opening induction shocks were always used, and the distance between the coils was always so great that the closing shocks were nearly, if not quite, without visible effect. There is always danger of radiation of current through the muscle, thereby causing the stimulus to start from a point nearer

the lever than is supposed. Somewhat stronger shocks than those actually used give no greater velocity, but for the sake of safety the weakest stimuli that would answer were used.

The wires from the secondary coil were so arranged that the cathode was nearest the lever. A commutator key was placed in this circuit, by which the stimulus could be sent in to any desired pair of electrodes, and also the stimuli produced in adjusting the signal short circuited off from the muscle.

Results.

Some variation occurred, but the results of different experiments agree quite as closely as could be expected, indeed more closely than any series of results that I have seen recorded. The variation in the different observations on the same muscle, too, is small when we consider how easily the condition of the muscle may be changed by fatigue, slight changes of temperature, etc.

In order to show more clearly the extent of the variations and upon what they depend, I give the details of a few experiments.

Experiment 6, March 1st, 1888.—Muscle carried 5 grams at each end. The secondary coil was 80 mm. distant from the primary. Temperature 26–27° C. Length of muscle from electrode to lever 62 mm.

Number.	Time.	Meters per second.
1	2.39	2.53
2	2.48	2.62
3	2.54	2.29
4	3.00	2.37
5	3.08	2.45
6	3.12	2.33
7	3.22	2.00
8	3.25	1.90

Experiment 7, March 5th, 1888.—Length of muscle traversed by wave 57 mm. Temperature 23–23½° C.

Number.	Time.	Meters per second.
1	2.40	2.00
2	2.46	1.96
3	2.50	1.81
4	2.55	1.81
5	3.18	1.86
6	3.21	1.81
7	3.25	1.78

Experiment 8, March 6th, 1888.—Length of muscle 57 mm. Temperature 24–24½.

Number.	Time.	Meters per second.
1	3.40	2.53
2	3.45	2.07
3	3.48	2.07
4	3.50	2.07
5	3.55	2.15
6	3.57	1.81

Experiment 11, March 9th, 1888.—Length of muscle 57 mm. Temperature 22¼–24½.

Number.	Time.	Meters per second.
1	10.15	2.18
2	10.21	2.10
3	10.25	1.78
4	10.29	1.78
5	10.32	1.43
6	10.35	1.45
7	10.38	1.41
8	10.40	1.47

These results show that the rate of the wave of contraction is quite constant in terrapin muscle, the maximum value observed being 2.62 and the minimum 2.00 meters per second. It will be noted, however, that in the experiments in which the rate found is highest, the temperature was somewhat higher. If this could have been more accurately controlled, the results of different experiments would undoubtedly have agreed more closely. It sometimes happened that the second determination would give a higher rate than the first. This was in all probability due simply to the fact that the muscle had not assumed the room temperature when the first measurement was made. Aeby found that the rate diminished very rapidly.[1] To give a few examples, he found that in one case the rate fell from 1.209 to .661 meter in 33 minutes. In another case it fell from 1.596 to .942 meter in 23 minutes.

In my results the same general condition is observed, but the decrease in rate is not nearly so rapid. In one case a velocity of

[1] l. c. p. 41.

.91 meter was observed in a muscle excised for 3½ hours, although not stimulated very frequently during that period.

Some interesting relations are found to exist with regard to the manner of applying the stimulus. von Bezold's experiment has been already described (p. 134). The conclusion which he drew was that in the intra-polar region the stimulus started at one pole and passed on to the other. Aeby varied this by placing the muscle on a horizontal surface with two levers resting upon it, and stimulating from end to end.[1] He found that *whatever the strength of current*, both levers rose simultaneously; hence he concluded that while one pole might be in a state of greater excitement than the other,[2] the whole intra-polar region was stimulated at the same time. In these two experiments the constant current was employed. Bernstein stated that the same was true when induction shocks were used. He showed this in two ways: first, he stimulated the muscle at the far end twice in succession, but with the current in opposite directions. The two tracings agreed perfectly, showing that in both cases the wave started from the same point. Second, he stimulated from end to end, and found that the lever rose at the same time whether the cathode was immediately under the lever or at the opposite end of the muscle.

This latter result I am unable to confirm. If the intra-polar region is very long (65 mm. was the actual distance), the lever always rose sooner when the cathode was immediately under the lever than when it was at the other end of the muscle. If the shocks be made very strong this relation will not be found, but the two curves will be identical. Radiation of current under these circumstances is certain, for to make the curves coincide, one must use stimuli vastly stronger than could with safety be employed in ordinary determinations of the velocity of the contraction wave. Under these circumstances, then, we must conclude that the stimulus starts from the cathode when induction shocks are used, and passes to the anode, but that its rate is vastly greater than in the extra-polar region.

In Plate XI, Fig. 7, is shown the graphic record of an experi-

[1] l. c. p. 59.

[2] Die Reizung der quergestreiften Muskelfaser durch Kettenströme. Arch. für Anat. und Phys. 1867, p. 710.

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ment of this kind, from which it appears that the time taken in traversing 65 mm. of intra-polar region is about .005 sec., corresponding to a rate of 13 meters per sec. This is very constant in different experiments.

In one more particular I am unable to confirm Aeby's results. Aeby states that the amount of load which the muscle carries has but slight effect upon the velocity of the wave. He gives the following figures for frog muscle: 1.264 with 5 grams load, 1.349 with 35, and 1.321 with 100.[1]

In Plate XII, Fig. 8, is shown a series of tracings in which the muscle was loaded alternately with 5 and 75 grams. From this it follows that the effect of a heavy load is to greatly increase the velocity of the wave, and very often indeed there is no wave. In another experiment, in which the muscle was not perfectly fresh, I find the rate 1.5 meters with 5 grams load, 2.1 with 25 grams, 9.2 with 50 grams. With 75 grams there was usually almost perfect coincidence of the two curves, but results gotten with heavy loads were always somewhat irregular, owing to the more profound changes in the muscle substance. The actual time intervening between stimulation and the raising of the load may be just as great as when the load is lighter, but the difference is in the latent period. This is very greatly prolonged.

The velocity of the wave of contraction then is greatly increased when the muscle is under considerable tension, and often there is no wave at all. The contraction does not begin until the muscle has gathered up sufficient energy to move the load, and then either the wave begins at the point of stimulation and travels along the muscle at a very rapid rate, or the whole muscle is simultaneously excited.

DESCRIPTION OF PLATES.

PLATE X.—Interruptor used in experiments on tetanus.

PLATE XI.—FIG. 1. Key *b* of Plate X, front and side views.

FIG. 2. Recording apparatus. *m*, connection with muscle. *a*, writing point.

FIG. 3. Sectional view of metal cylinder used as moist chamber, showing arrangement of muscle, electrodes, etc.

[1] Untersuchungen über die Fortpflanzungsgeschwindigkeit, etc., p. 40.

FIG. 4. Modification of Helmholtz "frog-interruptor" for determining the velocity of the contraction wave.

PLATE XII.—FIG. 1. Tetanic curve from terrapin muscle at 4° C. The middle line was produced by a tuning fork making 50 double vibrations per second. The lower line shows the rate of stimulation, which in this case is slightly less than 1 opening shock per second.

FIG. 2. Tetanic curve from sartorius of frog at 25½° C. 35 opening shocks per second.

FIG. 3. Tetanic curve from gastrocnemius of frog at 25½° C., produced by 37 stimuli per second. The two lower lines are omitted from this and succeeding curves for lack of space.

FIG. 4. Tetanic curve from terrapin muscle at 28° C. 34 stimuli per second.

FIGS. 5 AND 6. Tetanic curves from terrapin muscle stimulated with very weak induction shocks. Curve 5 was produced by 8½ stimuli per second; curve 6 by 13. The shocks were weaker in the latter case. The stimulation lasted longer than one revolution of the drum. The irregularity at *b* is owing to the drum being lowered.

FIG. 7. Portion of a tracing taken on a myograph plate, showing the rate of the contraction wave in the intra-polar region. The writing point leaves the base line sooner when the cathode is under the contact lever than when the electrodes are reversed.

FIG. 8. Effect of heavy loads on the velocity of the contraction wave. The muscle was loaded alternately with 5 and 75 grams (see page 144).

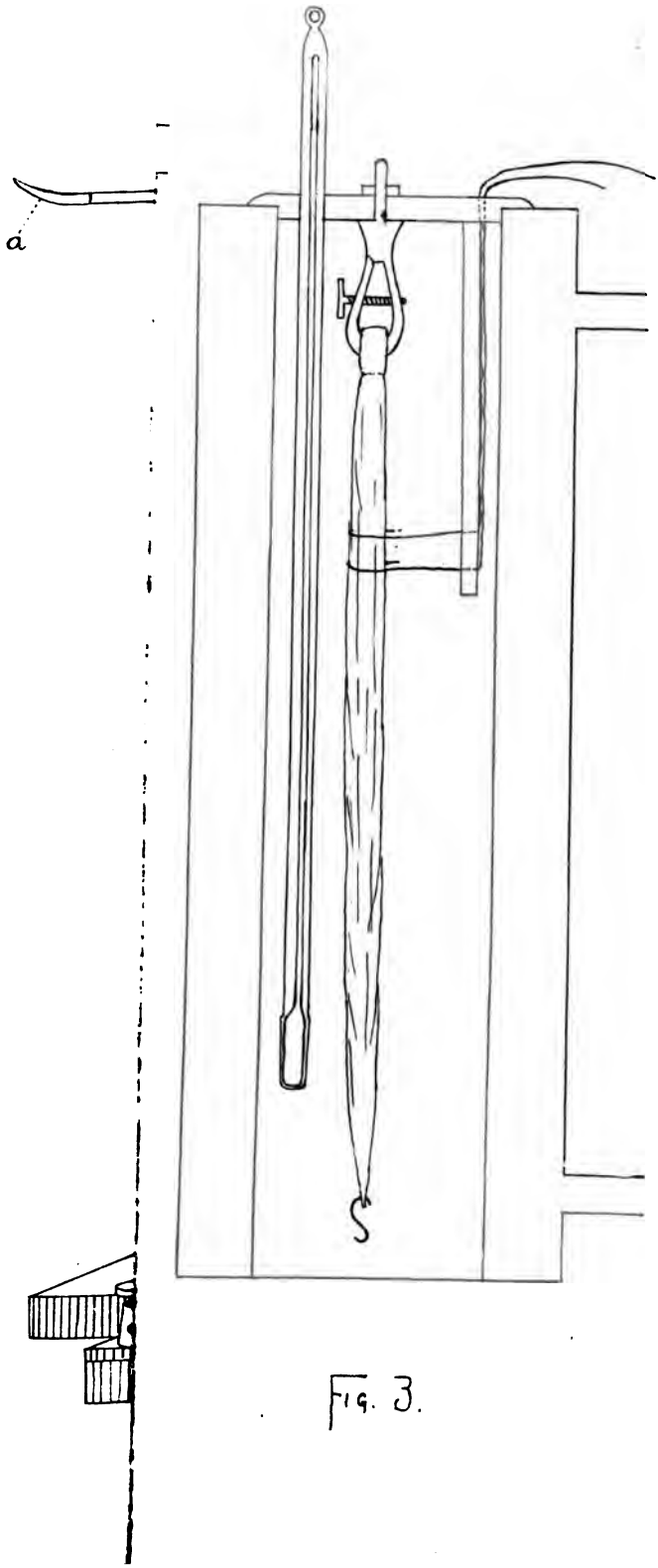
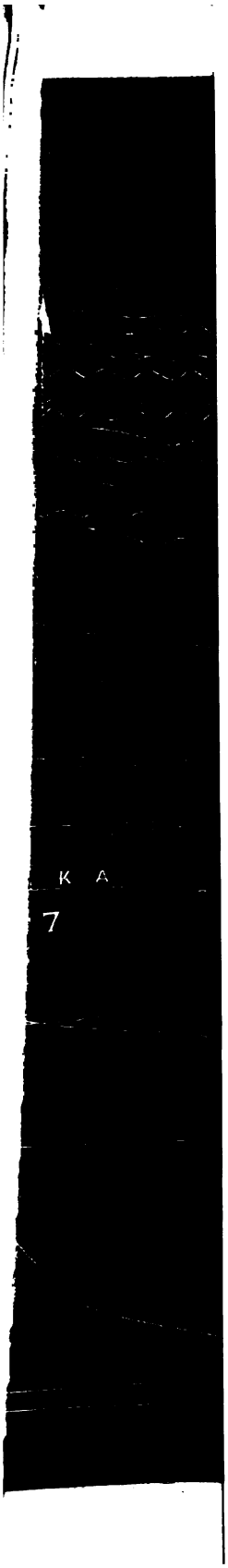


FIG. 3.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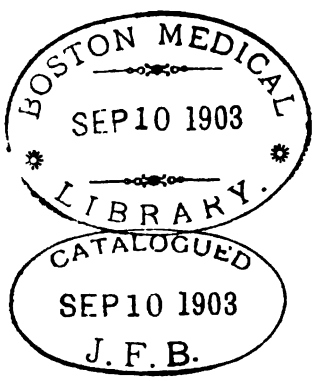

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The Life History of EPENTHESIS McCRADYI (n. sp.).

By W. K. BROOKS.

With Plates XIII, XIV, XV.

In June, 1887, I found at Nassau, N. P., in the Bahama Islands, a few specimens of a hydro-medusa belonging to the family Eucopidae (*Haeckel*), bearing upon each one of its four reproductive organs a number of hydroid blastostyles from which young medusae are produced by budding; a method of reproduction which has no exact parallel among the hydroids nor, so far as I am aware, anywhere else in the animal kingdom; for the production, by a medusa, of blastostyles which are morphologically equivalent to hydras, is a reversion, through asexual reproduction, to a past larval stage; a phenomenon which is thoroughly anomalous and exceptional.

In their endless diversity the hydro-medusae present nearly all imaginable permutations and combinations, but in all cases the life history of each species from the egg to the second generation of eggs is a history of development and progression. Hydroids like Eudendrium which bud off other hydroids; those like Podocoryne which bud off medusae, and medusae like Sarsia which bud off medusae, are all common and well known, but, so far as I am aware, no one has ever recorded, either in this group or anywhere else in the animal kingdom, the occurrence of buds which, like egg-embryos, recapitulate, in their own ontogenetic development, larval stages which their parent has already passed.

As I have already pointed out in a short preliminary paper,[1] the Nassau medusa is an exception to this general rule or law of reproduction, and I am now able to supplement this short notice by a more detailed and fully illustrated account of its remarkable history. The accompanying drawings show clearly that the bodies which are carried on the reproductive organs of the medusa are true blastostyles, and that the resemblance is no superficial one like

[1] "On a new method of multiplication in hydroids," University Circular No. 63, Vol. VII, Feb. 1888.

that which has been so frequently pointed out between the reproductive organs of medusae and medusa-buds. No one has ever questioned the homology between blastostyles and hydranths, nor, so far as I am aware, has any one disputed the correctness of the view which I have discussed at length in a paper on the "Life History of the Hydro Medusae," that the hydranth is essentially a medusa-larva. We therefore have in this case a recapitulation of larval stages without sexual reproduction.

In my first paper in the University Circular I spoke of the medusa as an Oceania, since it does belong to the genus which American zoologists are in the habit of designating by this name, although it is necessary to point out that it is not an Oceania as the word is used by foreign writers, but an Epenthesis.

In 1857 McCrady established the genus Epenthesis to include a new medusa of which he had found, at Charleston, S. C., a single specimen which he called *Epenthesis folliata*. He found no more specimens, and he gives no figures of the species, but his description is so clear and graphic that I have had no difficulty in identifying as *Epenthesis folliata McCr.* a medusa which is very abundant at all stages of growth at Beaufort, N. C. Although no figure has ever been published, my own notes contain a series of drawings of this species, showing the hydra, the blastostyle, the medusa when just set free, and many stages in its growth. The Nassau species is so very similar at all stages in its life to the Beaufort *E. folliata* that I do not hesitate to place it in the same genus. It presents certain differences which are usually regarded as of generic value, but as the Beaufort species is highly variable, and often varies in the features of difference from the Nassau species, it does not seem advisable to create a new genus for it.

As a slight tribute to the memory of my instructor, Professor McCrady, who established the genus Epenthesis, I propose for this remarkable species the specific name McCradyi.

At Nassau in 1887, and at Green Turtle in 1886, I found great numbers of small medusae ranging in size from about one twenty-fifth of an inch up to about one third of an inch, or a little less than the ones which carried blastostyles, these being nearly half an inch in diameter. The smaller medusae were like the large ones in every respect except that they had no blastostyles, and

they were very abundant, while only a few of the larger ones were found. There can be little doubt as to their identity, and it is probable that the smaller medusae are set free from hydranths in shallow water near shore, and are swept out to sea and scattered before they become fully grown and give rise to blastostyles.

On the algae in the harbors of Green Turtle and Nassau I found in great abundance a small campanularian hydroid, extremely like the one from which I reared *Epenthesis folliata* at Beaufort. When kept in aquaria these set free hundreds of medusae like the smallest ones which were captured at the surface, and while it is well known that the young medusae of various species of this family are often so much alike that their identification is a difficult and perplexing task, there can be little doubt that the hydroid is the larva of the medusae which were taken in the tow net.

EPENTHESIS McCRADYI (n. sp.).

(Plates XIII, XIV.)

Species Diagnosis.—Bell shallow, less than half as high as broad. Stomach very short, less than one eighth as long as the diameter of the bell, quadrate, with four simple, slightly recurved lips. Reproductive organs short, oval, about midway between the axis and the bell margin. Sixteen very long elastic tentacles, many times as long as the diameter of the bell, with enlarged hollow basal bulbs. Marginal vesicles varying in number from eight to sixteen, with a single osicle in each.

Ontogeny.—Larva a campanularian hydroid. Hydranths in cups with about twelve marginal teeth, on the ends of short erect slightly annulated unbranched stems from a creeping root. Blastostyles nearly sessile on root, in short annulated gonangia which are flattened distally. Each blastostyle carries three or four medusa buds which are set free in succession, the distal one being oldest. Medusa when set free nearly hemispherical, with four, sometimes eight, tentacles and eight marginal vesicles. Adult medusa nearly flat, with sixteen tentacles; with hydroid blastostyles in chitinous capsules on reproductive organs.

Size.—Fully grown medusa half an inch in diameter. Young medusa, when set free, about one forty-fifth of an inch in diameter.

Locality.—Hydroids and young medusae abundant in Nassau and Green Turtle harbors, Bahama Islands. A few specimens of the adult medusa found at Nassau in June, 1887.

Special Description.—Epenthesis folliata has sixteen marginal vesicles alternating with the sixteen tentacles, and Haeckel regards this number as diagnostic of the genus, but as I have found at Beaufort that this species is extremely variable, sometimes having three or two reproductive organs, and radial tubes, and occasionally having two stomachs and mouths, there is no reason why the marginal vesicles should be any more constant. One specimen of *E. McCradyi* had sixteen, alternating with the sixteen tentacles, and it was therefore an Epenthesis according to Haeckel's definition, although all the other adult specimens had, like the young, only eight, one on each side of the bulb of each inter-radial tentacle.

The species is easily distinguishable from *E. folliata* by the position of the reproductive organs. These are near the bell margin in *E. folliata*, and about half way between it and the axis in *E. McCradyi*. The hydranths of *E. folliata* are borne on branching stems, while all those which I found at Nassau were terminal on erect unbranched stems. This is, however, a point of little value, for the stems of young colonies of the hydroid of *E. folliata* are also unbranched, and under favorable conditions for growth it is quite possible that the hydroid of *E. McCradyi* may also branch.

The reproductive calycles of *E. folliata* are not annulated, and they are elongated in cross section so that they have two broad and two narrow surfaces. Those which are carried by the medusa of *E. McCradyi* resemble in these respects those which are found on the hydroid community of *E. folliata*, while those on the hydroid of *E. McCradyi* are annulated and are circular in cross section.

The lower figure in Plate XIV shows the hydranth and reproductive calycle of *E. McCradyi*. The upper figure in the same plate was drawn from a medusa which I reared in an aquarium from the hydroid from which the lower figure was drawn. The upper figure in Plate XIII is an oral view of the same medusa, and the lower figure in Plate XIII represents an oral view of one radial canal and about one fourth of the bell margin of an adult male

medusa, one half inch in diameter, with four blastostyles. At the time of its escape from the hydroid gonangium the medusa has a deep hemispherical bell about one forty-fifth of an inch in diameter, four fully developed radial tentacles, and four inter-radial tentacles which are sometimes rudimentary, sometimes fully developed at this age. On each side of the base of each inter-radial tentacle, and much nearer to it than to the radial tentacle, there is a marginal vesicle with a single osicle, or eight marginal vesicles in all.

Three days after it is set free the medusa, shown in oral view in Plate XIII and in profile in Plate XIV, has eight fully developed tentacles, the bell has become shallow, and traces of the reproductive organs have made their appearance on the radial canals. The older medusae are still more flattened and they have sixteen tentacles.

The reproductive calycles of the medusae are not annulated, they are slightly curved, and they are much wider in profile view than they are in symmetrical view. Their distal ends are flattened. Each blastostyle, like those in the hydroid colony, carries three or four medusa buds, which escape in succession. When set free the medusae are like those which are set free from the hydroid colony. All the medusae which I found with blastostyles were males.

The Structure and Origin of the Blastostyles of Epenthesis McCradyi.

I shall show in the sequel that the genus Epenthesis presents other interesting anomalies in asexual multiplication, and this fact gives a peculiar interest to this species. I have therefore made a careful study of the Nassau specimens by sections, and Plate XIV gives the details of the structure and growth of the blastostyle so far as it is shown by my material.

The topography of all the figures will be intelligible by comparison with Figure 1, in which *a* is the gelatinous umbrella of the medusa, *b* the sub-umbrella, *c* the cavity of the sub-umbrella, *e* the reproductive organ of the medusa, *d* the cavity of the radial canal, *k* the stomach of the blastostyle, and *i* its chitinous perisarc.

When I examined the living medusae at Nassau I thought that

the blastostyles preserved the continuity of the parental germ layers, and that their stomachs and those of the budding medusae were diverticula from that of the parent medusa, communicating with the radial canal and ultimately with the stomach through the cavity of the stem. This rule holds, as is well known, in all other examples of budding in the coelenterates, but the sections show that Epenthesis is an exception, and that the digestive cavity of the blastostyle is a new and independent chamber, without either direct or indirect communication with the exterior.

Figure 1 shows that while the ectoderm of the blastostyle is continuous with that of the reproductive organ of the medusa, the endoderm is not; and the food which is contained in the stomach of the blastostyle is not derived from the parent stomach, but from the tissue of its reproductive organ, upon which the blastostyle is a parasite.

This peculiar condition of things is shown in Figure 1, which passes through the axis of a young blastostyle of about the same age and size as the smallest of those which are shown in Plate XIII. In Figure 1, as in all the other figures, *a* is the gelatinous substance of the umbrella; *b* is the ectodermal epithelium of the sub-umbrella; *d* is the radial canal of the medusa, and *e* is the reproductive organ. At the base of the blastostyle its ectoderm *l* is reflected out on to the reproductive organ, and is directly continuous with the ectoderm of the medusa, *c*, of Figure 1, *f* of figures 2, 3 and 4. The endoderm *m* of the blastostyle separates from the ectoderm at its base, and penetrates the substance of the reproductive organ, where it forms a system of branching tubes *n n n*, which are nowhere continuous with the endoderm *h* of the medusa.

The Blastostyle.—The body of the blastostyle is divided into four regions: 1st, the system of branching tubes *n n n*; 2d, an enlarged thick-walled stomach, *k*; 3d, an elongated thin-walled "neck," and 4th, an enlarged knob-like "head." Of these four regions, all except the first are enclosed in a chitinous gonangium *i*, which is in close contact with the ectoderm of the head, widely separated from the neck, and in contact again with the ectoderm of the stomach *k*. At the point where the blastostyle joins the reproductive organ the chitinous perisarc fits into a deep circular furrow, in which it ends abruptly, so that no part of the ectoderm

of the medusa has a chitinous cover. The ectoderm of the stomach forms, like that of the reproductive organ, a columnar epithelium, and from the outer surface of the collar which is thus formed around the blastostyle, the perisarc, *i*, is excreted. The ectoderm of the "neck" forms a thin layer of flattened cells, each with a central thickening around the nucleus, and a very thin peripheral area. In this region of the body there are no cell outlines, but the protoplasm of adjacent cells is continuous. In the region of the "head" the ectoderm abruptly changes its character completely. Here the cells of both layers are "chorda cells" with a sharply defined, double contoured cell wall, enclosing a spacious cavity, inside which the nucleus, imbedded in a protoplasmic capsule, is suspended by a reticulum of fine radiating protoplasmic threads.

The ectoderm of the reproductive organ is columnar and very similar to that which lies on the base of the blastostyle, and except for the deep furrow which is occupied by the free or growing edge of the perisarc there is no change in the character of the ectoderm to mark the place where the blastostyle ends and the medusa begins. The surface of the reproductive organ has thickened ectodermal areas or ridges when this layer is more than one cell thick, but it will be seen by an examination of Figure 3 or Figure 1 that there is no constant relation between these ridges and the blastostyles. At the base of the reproductive organ the character of the ectoderm changes abruptly, and it is reflected outward at an acute angle to form the flattened epithelium of the sub-umbrella (Figs. 1 and 3 *b*), which is made up of cells like those which compose the epithelium of the "neck" of the blastostyle.

The Endoderm.—In the head the endoderm consists of "chorda cells" like those of the ectoderm, except that the protoplasm contains small spherical particles which grow more numerous near the neck, within which they become so numerous as to completely fill the cells. These bodies are not algae but food particles, and in some of the sections similar particles were found in the cavity of the head, together with very small granules as well as swollen and disintegrated cells and nuclei. In the stomach *k* of the blastostyle the endoderm cells are more columnar than they are in the neck, and they are quite similar in appearance to the endodermal lining, *h*, of the radial canal, *d*, of

the medusa. The nuclei, which are hidden in the neck by the opaque granular matter, are easily visible in the stomach, where they occupy the central or free ends of the cells. The peripheral ends are vacuolated and the vacuoles obscure the cell outlines, which are quite distinct at the central ends. Some of the vacuoles are clear and transparent, while others contain food particles surrounded by clear spaces. The endoderm cells of this region, like those of the neck, are very granular, and are packed with opaque particles ranging in size from the finest granules up to bodies about as large as the nucleus of the cell. As the endodermal tube leaves the body of the blastostyle and penetrates the substance of the reproductive organ of the medusa the cells become smaller, much more sharply defined, and the dark bodies, vacuoles and granules gradually disappear until at last the cells become perfectly transparent as shown at *n*.

In the substance of the reproductive organ the digestive cavity, which is constricted to a small tube at the base of the blastostyle, enlarges to form a chamber from which tubular endodermal roots are given off. These root-tubes, in some of the sections, contain more or less disintegrated cells and nuclei and granular matter, and if a root is traced through a series of sections it will at last be found to split along one side and to open out as shown in Fig. 2, *n*, in such a way that the central cavity—a diverticulum from the digestive cavity of the blastostyle—is covered on one side by the endoderm of the blastostyle and on the other by the tissue of the reproductive organ of the medusa. At these points the tissue of the reproductive organ undergoes degeneration; the outlines of the cells become indistinct and the cells themselves become detached and pass into the cavity, where they gradually break up into granular matter and detached nuclei, like those which are found in the vacuolated endoderm cells of the stomach and neck of the blastostyle. Figures 1 and 2 represent two parallel sections from the same specimen, separated from each other by six intervening sections. The two tubes which are distinct from each other in the upper part of Fig. 1, have united in Fig. 2 to form the large chamber, which is incomplete, or open along its central side, where its wall is formed by the degenerating cells of the reproductive organ. In the next section from the the same specimen the endoderm of the wall of the tube merges

with the cells of the reproductive organ and disappears, while in sections on the other side of the one from which Fig. 1 was drawn, the lower one of the two tubes shown in this figure communicates with the endodermal chamber at the base of the blastostyle. None of the sections showed any connection between the endoderm of the medusa and that of the blastostyle, and the food found in the latter is entirely derived from the substance of the reproductive organ upon which it subsists as a parasite.

The radial canal of the medusa is dilated in the reproductive organ to form a capacious pouch, *d*, figures 1, 2, 3 and 4, the walls of which are made up of a single layer of endoderm cells similar to but somewhat larger than those which form the stomach of the blastostyle. The central chamber was empty in all the sections, but all the endoderm cells, except those which form the roof of the aboral end, are much vacuolated and granular. The peripheral ends of the cells are so honeycombed by the vacuoles that they are reduced to a network of protoplasmic threads with no cell outlines. The deep-seated vacuoles are filled with a clear fluid, but most of those near the free ends of the cells contain foreign bodies.

The Supporting Layer.—The ectoderm of the reproductive organ of the medusa is bounded internally by a well defined supporting layer which lies between it and the substance of the reproductive organ. At the point where the latter is suspended from the umbrella the supporting layer divides into two portions, of which one bends outward at an acute angle to form the supporting layer of the sub-umbrella, while the other forms a thin stratum between the gelatinous substance of the umbrella and the endodermal roof of the radial canal. This latter is the only portion of the endoderm of the reproductive organ which is directly bounded by a supporting layer.

The ectoderm of the blastostyle is separated from the endoderm by a supporting layer which is easily seen in all parts of the blastostyle. At its base it divides into two portions, of which one is continuous with that which lies between the ectoderm of the medusa and the reproductive tissue, while the other forms a basement membrane for the cells of the endodermal tubes.

The Reproductive Organ.—All my specimens were males. The reproductive tissue which lies on the inner or endodermal

side of the supporting layer and is not sharply separated from the endoderm, consists of cells of three kinds: The germ cells, Fig. 4, *o*, which are probably of endodermal origin; the male cells, Fig. 4, *p*, which are probably derived from the larger cells *o*, and the groups of cells shown at *q* in Fig. 3. The germ cells *o*, which in all the sections form the greater part of the reproductive organ, are polygonal cells, smaller than the endoderm cells but larger than the ectoderm cells, with large nuclei suspended in a protoplasmic reticulum, which also forms a granular sheath around the periphery of the cell by means of which the outlines of the cells are marked out distinctly, although there are no sharp lines of demarcation to separate adjacent cells from each other. The use of colors in the figures to distinguish the different layers from each other gives an unnatural sharpness to the line which separates these cells from the endoderm. In the specimens there is no such distinction between them, and while I found in my sections no endoderm cells undergoing division, the relation of the cells to each other seems to indicate that the germ cells have arisen by the division or migration of the true endoderm cells, from which they are not separated, as they are from the ectoderm, by a supporting layer. It is possible that the primary germ cells are derived from the endoderm at an early stage in the life of the medusa, and that they afterwards multiply by division among themselves without any new addition from the endodermal epithelium.

The male cells, *p*, figures 3 and 4, form small groups or clusters of very small cells which are scattered among the larger cells at intervals throughout the whole substance of the reproductive organ and also between the peripheral ends of the endoderm cells. They stain much more intensely than any other part of the section and are therefore conspicuous. Each group consists of a number of nuclei, much smaller than those of the germ cells, packed close together in an investing mass of very granular protoplasm in which no cell outlines could be made out. Scattered through the substance of the reproductive organ near its abaxial end, that is the end nearest the bell-margin or farthest from the stomach, there are groups of cells, Fig. 3, *q q q*, of about the same size as the germ cells, from which, however, they differ in several particulars. Their outlines are sharply

defined, and they are arranged side by side in an epithelium. As the cells themselves are wedge-shaped with the broad end of the wedge nearest the surface of the reproductive organ, the epithelium is not flat but convex on its outer surface. I found one group which consisted of only two of these cells, and many other groups with three, four or more. In the larger groups there is an empty space on the central surface bounded internally by the cells of the reproductive organ, and externally by the epithelial cells, which are the endoderm cells of new blastostyles. As regards the origin of these cells my sections give no decisive evidence. They are in all probability derived from the germ cells, but I have seen nothing to indicate whether each group originates in a single cell which divides and thus builds up the group, or whether the group grows by the addition of new germ cells to its edges.

Metschnickoff has recently described the parthenogenetic multiplication of Cunina larvae by what he terms "sporogenesis." In Cunina the embryonic germ cells of both male and female specimens may give rise by a process of segmentation to an embryo consisting of two germ layers, but before this process of parthenogenetic development begins, each one of the germ cells which is destined to become an embryo becomes inclosed in another which serves as a nutritive apparatus. In the case of Cunina we have, in all probability, simply an example of extremely precocious development of germ cells by the ordinary process of segmentation, and the whole body of the new organism is descended from a single germ cell just as the body of an ordinary metazoan is descended from a fertilized egg.

It is probable that Epenthesis also is an example of sporogenesis, and that the endodermal tube is derived from a single cell by segmentation, but this is certainly not true of the ectoderm of the blastostyle, and if we have sporogenesis at all in Epenthesis we have it in combination with budding.

The Production of New Blastostyles.—None of my specimens furnished anything like young blastostyles growing from the stems of older ones, nor have I found any young buds growing upon any of the root-tubes which join the bodies of larger blastostyles. So far as the evidence of my scanty supply of specimens goes, each blastostyle, with its own system of root-tubes, is

quite independent of all the others. It gives rise to no buds except those which are to become medusae, and it is itself formed as an independent structure on the reproductive organ of the medusa. A group of cells like those which are shown at *q* in Fig. 3 gradually increases in size, and its convex external surface comes into contact with the supporting layer of the reproductive organ, while its concave central surface becomes hollowed out, thus giving rise to a chamber or cavity which is bounded internally by the tissue of the reproductive organ. The section shown in Fig. 4 shows a young blastostyle at a somewhat later stage of development. A bud-like process *k* grows out from the centre of the endodermal area to form the endoderm of the blastostyle, and carries with it an investing layer of ectoderm cells, directly continuous with and at first perfectly similar to the ordinary ectodermal epithelium of the reproductive organ of the medusa. Soon, however, these ectoderm cells which enter into the structure of the bud excrete from their exposed ends a thin transparent cuticular layer filled with fine granules, and as this excretion thickens and becomes free from the ends of the cells it becomes the perisarc. The endoderm of the blastostyle is derived from the central cells of the endodermal area, while its peripheral cells become the root-tubes. The digestive cavity of the blastostyle is a diverticulum from the cavity shown in Fig. 3, and, as shown in Fig. 4, it, while still very small, is filled with the detritus of the germ cells which are set free and disintegrated during its formation.

The origin of the medusa buds on the blastostyle presents nothing exceptional. They are formed as they are in ordinary campanularian hydroids, and there is nothing to distinguish them from the medusa buds which have been so minutely described by many writers. My series of sections show them at various ages and in different planes, but it did not seem to be worth while to draw them. My material did not enable me to trace the origin of the male cells of the medusa buds, but as the sections of all the young blastostyle buds showed a group of male cells lying in contact with that part of the supporting layer which is subsequently drawn out into the body of the blastostyle (see Figs. 3 and 4), it is probable that some of these cells migrate, with the endoderm, into the blastostyle, and subsequently pass into the medusa buds.

SUMMARY.

The medusa of *Epenthesis McCradyi* carries on its reproductive organs campanularian hydroid blastostyles, inclosed in chitinous gonangia.

The blastostyles do not multiply by budding or form hydroid cormi, although they produce medusae by budding.

The ectoderm of the blastostyle is produced by ordinary gemmation, and it is directly continuous with the ectoderm of the medusa.

The endoderm of the blastostyle has no direct connection with that of the medusa, although the germ cells from which it is derived are probably endodermal in their remote origin. These germ cells give rise to the endoderm of the blastostyle by a process of specialization which is very similar to what Metschnickoff has described in Cunina and has termed *sporogenesis*.

The blastostyles and medusa buds receive no food directly from the stomach of the medusa, nor do their stomachs communicate with any part of its chymiferous system. They are parasites upon the tissue of its reproductive organ.

Asexual Multiplication in the Genus Epenthesis.—Medusae which multiply by budding are by no means uncommon, and in some families nearly all the genera and species are occasionally found with buds. While proliferous medusa are somewhat rare near shore and in land-locked waters, they are familiar objects to all naturalists who have studied the fauna of the open ocean, and buds are known to occur in a large percentage of the strictly pelagic species of hydro-medusae.

The Eucopidae, the family to which Epenthesis belongs, is not, however, among those in which proliferous medusae are common, for although it is a large family, including many pelagic species, it is among the few in which budding is almost unknown.

Haeckel says (System der Medusen, p. 166) that it has never been proved to occur in any species of the family, and that while there are on record a few statements to the effect that specimens of medusae belonging to this family have occasionally been found with buds on the stomach or on the radial canals, he believes that these statements are based upon untrustworthy observations or upon accidental monstrosities.

We have, however, in *Epenthesis McCradyi*, a medusa of this family with buds, and it is very probable that another species, *E. folliata*, multiplies asexually by fission; each medusa undergoing segmentation into two, each of which ultimately repeats the process.

Lang has recently published (Jen. Zeitschr. XIX, IV, 736) a most interesting account of a medusa which multiplies by fission, and also by incomplete fission ultimately builds up a complex polystomatous medusoid cormus. He gives a minute account of the process of division and illustrates it by a series of drawings of the successive stages.

At Beaufort I have found specimens of *Epenthesis folliata* which in all essential particulars duplicate Lang's figures, and my notes contain a drawing made at Beaufort in 1881, or five years before his paper was published, from a specimen almost exactly like his Fig. VIII, B. 1, Plate XXI. It has only two radial canals, and these meet at an obtuse angle in a large stomach, near which on one of the canals is a much smaller stomach also with a mouth.

It has more tentacles than the specimen which he figures, but the resemblance is so perfect in other respects that it is clearly one of the products of an act of fission like that which he describes, and we must therefore add *Epenthesis folliata* to the list of gastroblastic medusae.

Many years ago Keferstein gave a figure (Zeit. f. Wiss. Zool. XII, p. 28, Taf. II, 9 and 10) of a medusa which, from the possession of what he regarded as a bud on the stomach, he called *Eucope gemmigera*. Haeckel has shown that it is not a Eucope at all, but a representative of McCrady's genus Epenthesis, *E. cymbaloidea Haeckel*.

In his description Keferstein says that the continuity of both the layers of the bud with those of the parent was plainly recognizable, and although Haeckel, who has had ample opportunities to study the same species, confidently expresses his belief that the body which is shown in the figure is not a bud at all, and that no authentic case of budding is known in the Eucopidae, the fact that *Epenthesis folliata* is sometimes polystomatous renders it at least possible that Keferstein may have found a specimen with a second stomach.

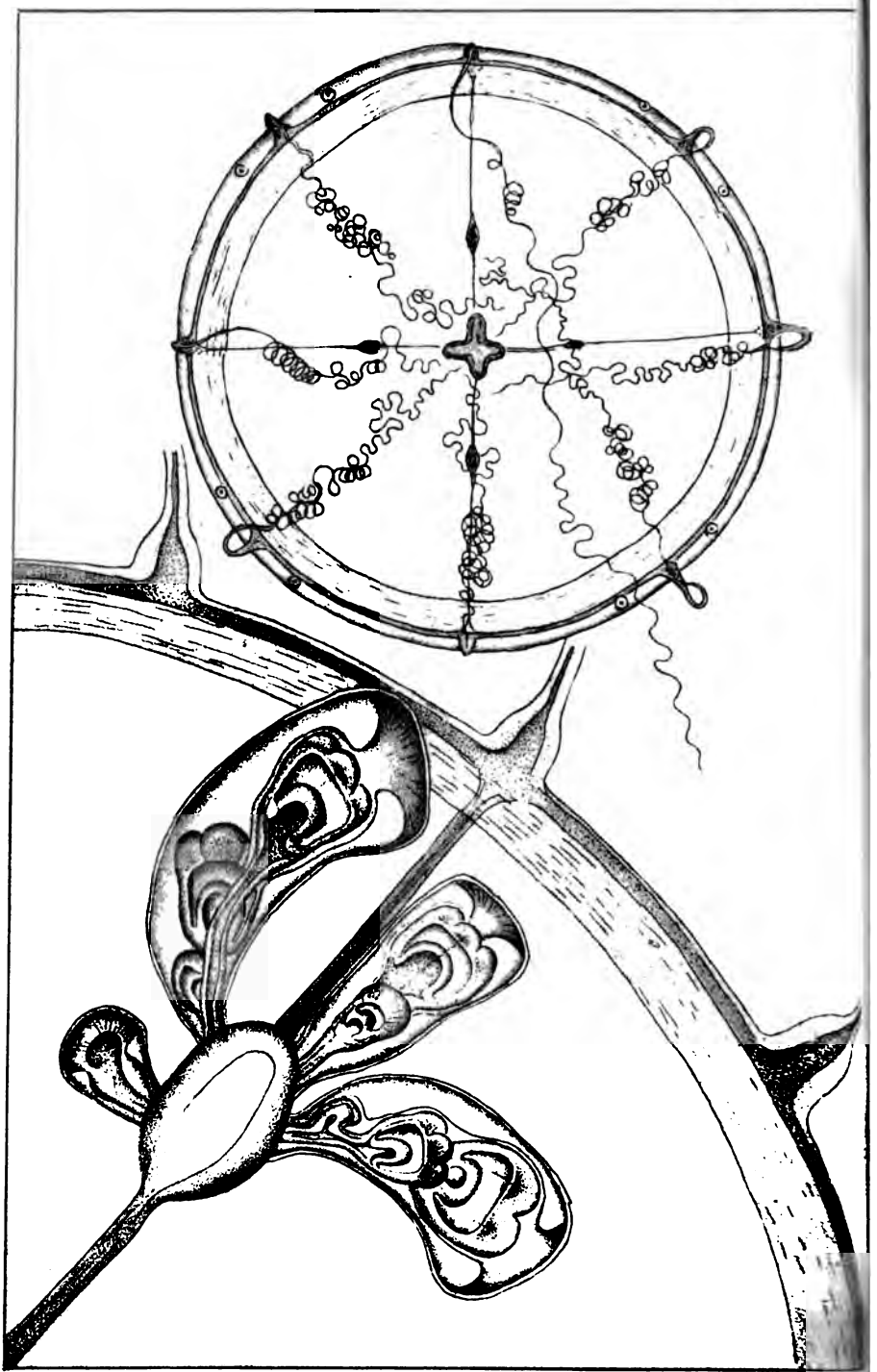
There is no reason for supposing that fission is restricted to a single genus, for if it exists in one pelagic medusa it may also occur in others, and so far as the genus Gastroblasta is based upon this characteristic there is no reason for recognizing it. I think, however, that the study of Lang's figures will show that his species, *Gastroblasta Raffaelii*, may possibly be an Epenthesis, for although no other species of the genus is known to possess centripetal canals or more than sixteen tentacles, the replacement of canals by centripetal growth from the radial canal is obviously an adaptation which has been acquired to meet the conditions which result from fission, and the normal or typical *Gastroblasta Raffaelii* would seem, from Lang's account and figures, to be a medusa with four radial canals and sixteen tentacles, alternating with sixteen sense organs; that is, an Epenthesis.

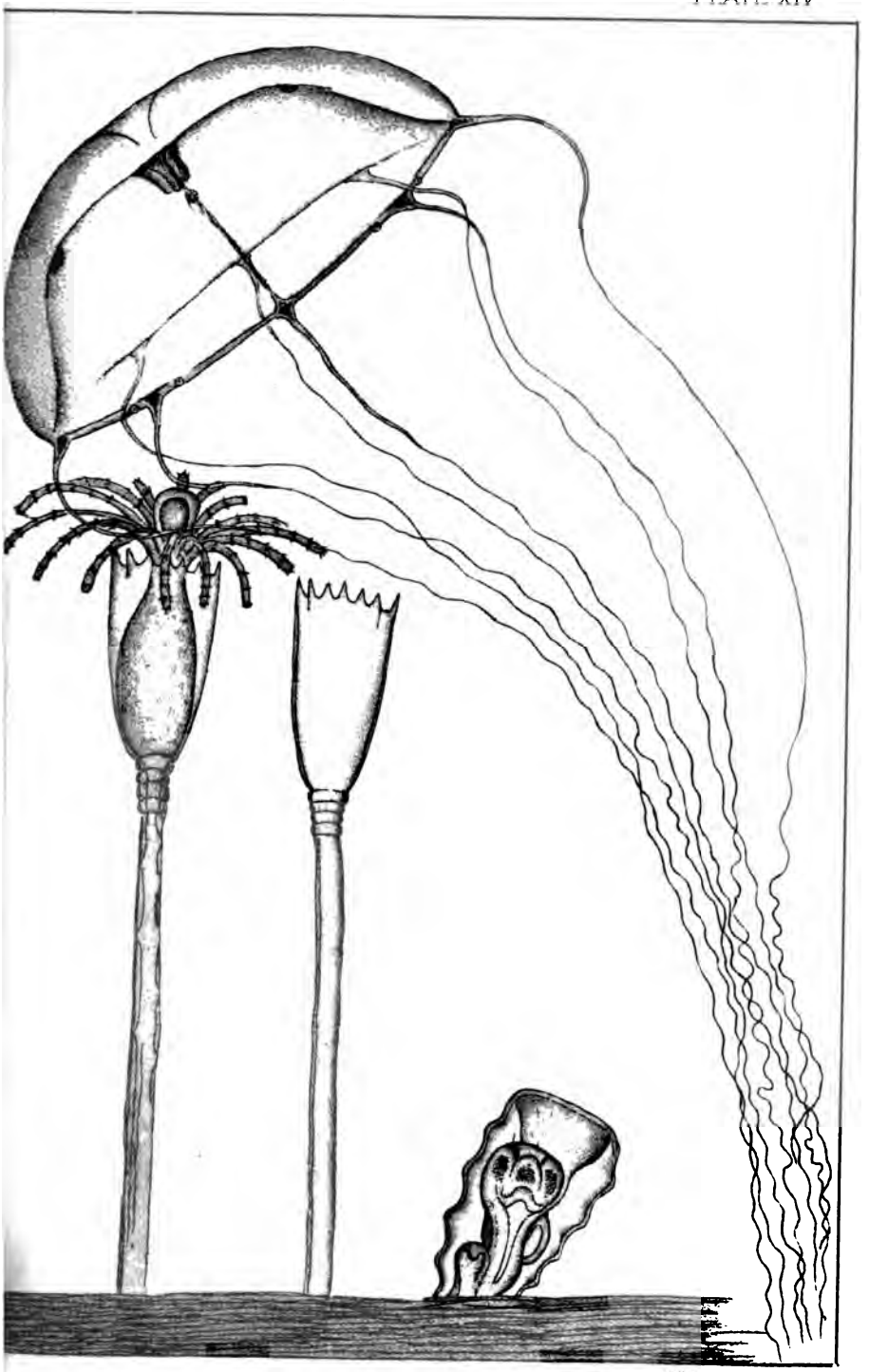
As regards its habits, its rapacity, its mode of locomotion and its tendency to roll together along one diameter, Lang's description would perfectly fit *Epenthesis folliata;* and while he gives no figure of a form with sixteen tentacles, the diagram which he gives in Fig. 1 of Plate XXI as the imaginary type for his species is in all particulars a young Epenthesis, and if the number of tentacles and sense organs in this diagram were doubled we should have a perfect Epenthesis as the genus is defined by Haeckel and McCrady.

It is difficult to decide whether Keller's *Gastroblasta timida* (Zeit. f. Wiss. Zool. XXXVIII, 622, Taf. XXXV, Figs. 1–5) belongs to the same genus or not. Assuredly no Epenthesis has endodermal sense organs, and Keller's figures do not suggest Epenthesis, but except for his statement to the effect that the sense organs are endodermal, and also except for the great number of tentacles, his description would apply very well to Epenthesis.

Our knowledge of the transformations which are undergone by those medusae which are swept out to sea and drift among limitless supplies of food far from land or from any resting place for hydroids is very fragmentary indeed, and we know barely enough to be confident that under these conditions many of them increase and multiply asexually, and continue to perpetuate the species without the intervention of a sessile hydroid stage. We

know that in many species this pelagic life is most important, although the study of the complications which are introduced into the life history of the species during this stage is at present an unworked field. Those specimens of *Epenthesis folliata* which are carried out into the ocean probably multiply by fission. It is not improbable that *Gastroblasta Raffaelii* is also an Epenthesis which in addition to this power is also able to build up, by incomplete fission, polygastric medusae of considerable size. *Epenthesis McCradyi* presents still another method of solving the problem of multiplication without a hydra stage, and it produces by budding so much of the hydroid as is available at sea, that is the blastostyle, and this then gives rise to medusae. Lang has pointed out the physiological resemblance between the polygastric form of *Gastroblasta Raffaelii* and the Porpetidae and Velellidae, and has called attention to the fact that while we cannot regard Gastroblasta as a stage in the evolution of these Siphonophores, its existence does illustrate the way in which Porpita may have been evolved from a polygastric medusa. It is interesting to note that *Epenthesis McCradyi*, with its pendant blastostyles hanging from a swim bell, and carrying medusa buds, stands in a somewhat similar relation to the ordinary Siphonophores.





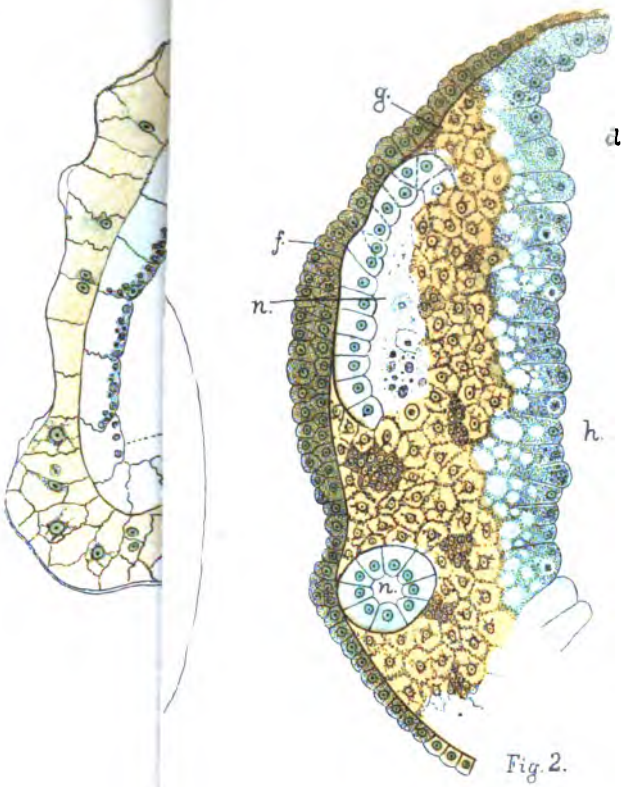


Fig. 2.

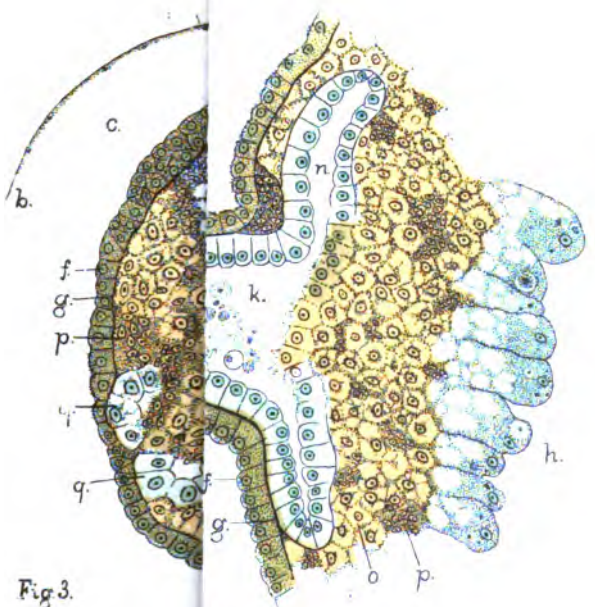


Fig. 3.

PHOTO-LITH BY A. HOEN & CO. BALTIMORE, MD

OBSERVATIONS ON THE DEVELOPMENT OF CEPHALOPODS; HOMOLOGY OF THE GERM LAYERS.

By S. WATASE. With Plates XVI and XVII.

I.

Notwithstanding the fact that the Cephalopods have been thoroughly studied by several observers, the formation of the germ layers requires further elucidation, inasmuch as the details of recorded facts admit the most diverse interpretations. The following observations on the development of the common squid and of some unknown pelagic Cephalopod were chiefly carried on with a special view to the formation of germ layers, in order to find out, if possible, from the study of this important point, definite homology in different embryonic layers in comparison with other groups of animals.

The extensive collection of the common squid (Loligo Pealii) which was made by the late Dr. A. T. Bruce contained the materials for the study of the very early stages, and I owe him my sincere thanks for generously placing it at my disposal for study and for the kind directions he gave me in the earlier part of my work. We originally contemplated a joint work upon the subject, but his sad death has thrown upon me the entire responsibility of completing it. The results which he had reached at the time when his work was interrupted are contained in his own paper ('86*b*).

I have also to acknowledge my thanks to Mr. H. T. Fernald for placing at my disposal different stages of some unknown pelagic Cephalopod as well as the sketches accompanying them. The specimens, however, were too far advanced for the study of the germ layers, although they contributed essentially to the study of the formation of the digestive tract and the ink-bag.

This Cephalopod was found on the beach of Nassau by a little daughter of Dr. Brooks, Menctta, when our Marine Laboratory was located at the West Indies during the summer of 1887. This very closely resembles the Gibraltar specimen which furnished the materials for Grenacher's well known memoir on the development of Cephalopods ('74*b*).

During the months of August and September, 1887, while enjoying the facilities for study offered by the table of the Johns Hopkins University at the U. S. Fish Commission Laboratory at Wood's Holl, Mass., I made a series of observations on the development of the common squid, especially in the later stages of growth. Different kinds of reagents for preservation were tried. The best preparations were obtained by immersing the whole bunch of egg capsules in diluted osmic acid (½ per cent), leaving them until the embryos showed dark brown color; washing them with distilled water and hardening them in different grades of alcohol. Pereny's fluid has produced very satisfactory results. The formation of the digestive tract in the squid was studied entirely from this collection. My best thanks are due to Dr. W. K. Brooks for valuable assistance in the course of my investigation as well as on the preparation of illustrations on stone.

The long series of observations which began with the remarkable contributions of Aristotle have given us a pretty complete acquaintance with the outline of Cephalopod development, and I will not repeat here those points which have but a secondary bearing on the origin of the germ layers. Before, however, examining the observations recorded by preceding observers, I will go over in a summary way the results obtained in my investigation, touching such points as bear directly on the question at issue.

The earliest stage I studied in the squid (Loligo Pealii) is one in which the germ protoplasm is divided into two segments, Fig. 1, Plate XVI. The protoplasm forms a thin cap over one pole of the huge yolk mass, and is differentiated into three zones which correspond to the three zones of later stages—thin in the centre, thicker in the intermediate, and thinner again in the peripheral zone. One noticeable feature in the distribution of the food yolk and the germ protoplasm is that the line of demarcation between the two is perfectly distinct from the beginning of segmentation. As a consequence of this the food yolk exerts no disturbing effect on segmentation. Furrows of segmentation pass through the entire thickness of the germ disk. The size of the segments, however, enlarges gradually as we proceed from the centre of the disk to the periphery, where the protoplasmic layer becomes thin and the segments assume pyramidal outlines with their bases turned towards the growing edges of the disk.

Meanwhile the thicker intermediate zone divides transversely, or by delamination gives rise to a zone of two cells thick. In this stage the germ disk consists of three zones, as has already been pointed out by Köllicker: (1) the central, a circular area, one cell thick; (2) the intermediate zone of two cells thick—*area opaca* of Ussow; (3) the marginal zone, one cell thick—blastocones of Vialleton.

In this stage also some cells in the lower layer of the marginal zone lying near the junction with the intermediate zone are seen to become spindle-shaped in cross section, showing the characteristics of the future "yolk membrane" cells, Fig. 2 *Hy*, Pl. XVI. The germ disk is in this stage a sort of inverted cup over the pole of the yolk mass, with its concave side turned toward the yolk. The "yolk membrane" cells originating at the inner side of the germ disk and at the periphery of the intermediate zone travel towards the centre of the disk, over the surface of the yolk mass from all sides, until finally they join with each other, with their anastomosing processes forming a complete network of plasmodium underneath the blastodisk. The original blastodisk is thus completely separated from the yolk mass by the intervention of the "yolk membrane" between them.

As the germ disk grows over the food yolk, the march of the "yolk membrane" keeps pace with the growth of the overlying layer, but always lying just inside of the growing edge.

This stage strongly suggests a gastrula, comparable in all essentials to the typical gastrula, making however a full allowance for the accelerating influence of abundant nutrition in the order of morphological development. The whole germ disk is an inverted gastrula with its concave side turned towards the yolk mass. The line of junction between the food yolk and the marginal zone corresponds to the lip of the blastopore, and the shallow cavity formed by the disk enclosing a portion of the food yolk corresponds to the archenteron. Viewed in this light, the first origin of the "yolk membrane" cells in the inner side of the marginal zone and their subsequent centripetal growth fully justifies a comparison to the lining of the gastrula cavity or to the endoderm. The delamination of the intermediate zone resulting in the formation of the mesoderm, Figs. 2 and 3 *Ms*, Pl. XVI, before the lining of the gastrula cavity is complete,

must be looked upon as a case of accelerated development, due probably to the influence of the food yolk.

By the successive delamination of the intermediate zone the mesodermic zone becomes many cells thick. The lip of the blastopore extends gradually over the yolk mass, the underlying "yolk membrane" inside the edge keeping pace with it, until finally the blastopore closes at the pole just opposite to one where the germ disk made its first appearance. At this stage, therefore, the pole where the germ disk first originated, consisting of three layers, ectoderm, mesoderm, and "yolk membrane," is the area where the embryo develops, while the pole just opposite to this, enveloped by the ectoderm and the "yolk membrane" only, is the area of the food yolk. In other words, the whole mass of the food yolk is completely enveloped by the two concentric layers, the ectoderm and the "yolk membrane," while the upper half of the same has in addition a third enveloping mass—the mesoderm—which fills the spaces between the ectoderm and the "yolk membrane."

The "yolk membrane" assumes exclusively the digestive function. The yolk spherules lying near this layer become divided into fine fragments. Later, this layer shows within itself two zones, the outer granular and the inner non-granular layer. This differentiation is more marked around the portion of the food yolk which lies in the inside of the developing embryo. The conspicuous mass hanging down from the embryonic portion is the bulk of the food yolk enveloped by the two concentric layers, the ectoderm and the "yolk membrane." The "yolk membrane" here shows but a slight differentiation within itself. The embryonic portion and the hanging yolk mass are connected by a rather narrow tract, the "waist" (Fig. III, p. 170). This waist becomes narrower and narrower with the growth of the embryo, showing thus the characteristic feature of many forms of Cephalopod embryos that gave rise to the remarkable comparison drawn by Aristotle, who likened it to the shape of a bird, whose head corresponds to the embryo and the belly to the hanging yolk sac. For the detailed account of the changes undergone by this form (Loligo Pealii) I must refer the reader to Brooks' memoir ('**80***a*).

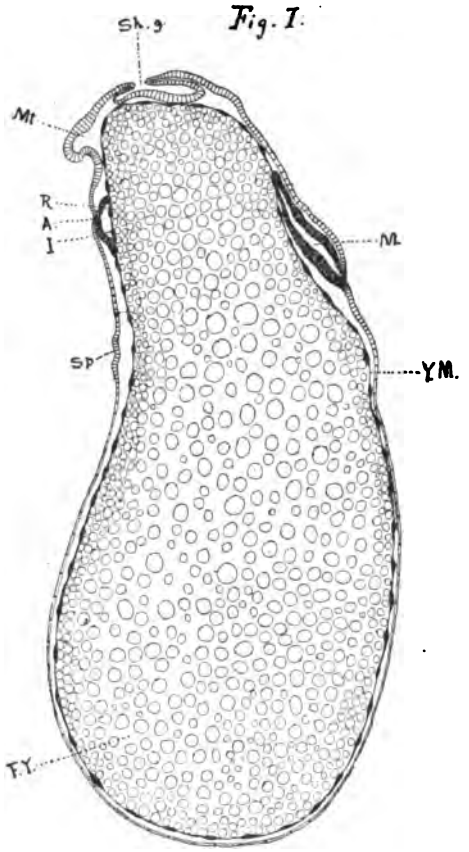
To return again to the germ layers. I recognize, then, in the early stages of Cephalopods the existence of three germ layers:

the outer columnal ectoderm, the middle polygonal mesoderm, and the inner "yolk membrane" or the endoderm.

The recognition of the "yolk membrane" as the endoderm in Cephalopods requires some evidence additional to that already given. The most important test would be to determine whether this "yolk membrane" takes any share in the formation of the digestive tract of the adult or not. In dealing with the development of the digestive tract I will confine myself to the formation of the proctodæum and its appendages only, for the stomodial invagination is so evident as to leave not the slightest doubt as to its history. Besides, Grenacher ('74*b*) has given such a thorough account of the stomodial invagination and its appendages in his Cephalopod that any further account is not here necessary.

Fig. I, p. 168, is the median section of a young squid embryo in which stomodial and proctodial invaginations have commenced to take place. The invagination of the stomodæum is so distinctly marked as to leave no doubt as to its occurrence. But the evidence for the proctodial invagination is so slight and of such a misleading character that great confusions exist regarding it.

The proctodial invagination, unlike the stomodæum, leaves no cavity of invagination at the surface, but the lips of the cavity come into close apposition. As the invaginated epiblast travels further inward and forms a relatively spacious cavity underneath the general surface of the body, that portion of the epiblast becomes elevated above the surrounding level, just between a pair of rudimentary gills. It seems as if the lower layer of mesoblast cells arranging themselves in that particular fashion and arching over the yolk mass, form the lumen of the digestive tract. But the examination of well prepared sections through this region shows the distinct line of continuity between the ectoderm and the walls of the rectum and the ink-bag, Fig. 9, Plate XVII. The arrangement of cells as well as of their nuclei in this section shows absolute continuity between the external ectoderm and the walls of the rectum and the ink-bag. The spaces between the ectoderm and the invaginated proctodæum are packed with mesoblast cells. Grenacher's Cephalopod shows a similar arrangement in this regard, Fig. 15, Plate XVII. Fig.



Explanation of the Diagrams.

Fig. I. The median longitudinal section of the squid embryo, in which the stomodial and proctodial invaginations have commenced to take place.

Fig. II. The same in a more advanced stage (p. 170).

Fig. III. The same in a still further advanced stage. The fore and hind gut have met at the apex of the food yolk mass, completing the whole digestive tract (p. 170).

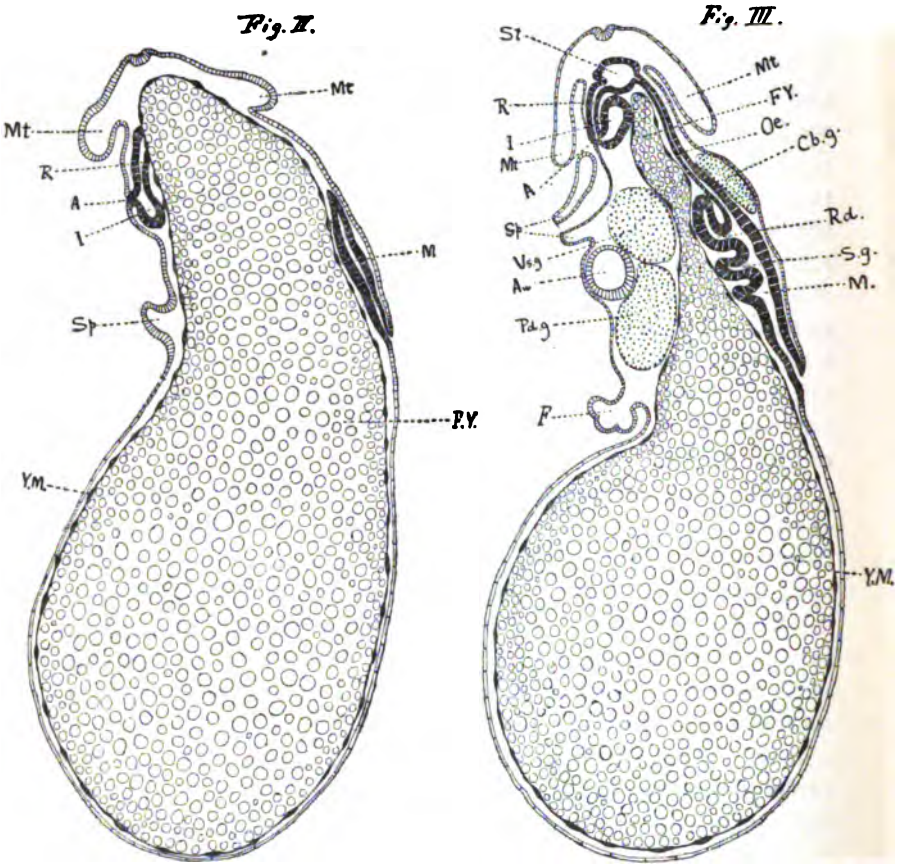
A., Anus.
Au., Auditory sac.
Cb. g., Cerebral ganglia.
F., Foot.
F. Y., Food yolk.
I., Ink-bag.
M., Mouth.
Mt., Mantel.
Oe., Oesophagus.
Pd. g., Pedal ganglia.
R., Rectum.
Rd., Radula sac.
S. g., Salivary gland.
Sp., Siphon.
St., Stomach.
Vs. g., Visceral ganglia.
Y. M., "Yolk membrane" (endoderm).

14 is another section which with Fig. 15 forms a series from the same individual. In this the plane of section does not pass through the point of invagination, and the walls of the proctodæum and the ectoderm simply come in contact. Fig. 10 (Loligo), Plate XVII, is a section through the region of the anal elevation in an individual of the same stage as Fig. 9. The plane of section passes a little on one side of the point of invagination. Between the ectoderm and the digestive tract *R'* there comes a distinct layer of mesoblast. These few examples show how easily we may be led astray in our interpretation of this point, for in absence of consecutive series it is quite easy to overlook the sections showing the connection between the ectoderm and the proctodæum which is seen but in one or two sections in the whole series. I feel confident that this mistake has been made and that it explains the lack of agreement between my observation and of those of some others. No doubt the process of hardening serves to assist in bringing the lips of the invagination into closer contact and exaggerates the obscurity of the original passage. Our only guide, therefore, to determine this point is the arrangement of the cells and the shape and position of their nuclei.

In the more advanced embryo, Fig. II, p. 170, the invaginated cavities extend further inward; the distinction between the rectum (*R*) and the ink-bag (*I*) is more apparent; the mesoblast cells around the ink-bag arrange themselves in a regular layer; the floor of the rectum which is continuous with the walls of the ink-bag travels dorsalward along the posterior border of the yolk mass over the "yolk membrane." This extension of the proctodial cavity continues further until finally the digestive tube mounts over the yolk mass and becomes continuous with the prolongation of the stomodial invagination, and thus the continuity between the mouth and the anus is completed, Fig. III, p. 170; Fig. 12, Plate XVII. The anus, which is quite indistinct in the stage Fig. 9, becomes more marked in Fig. 11, and remains permanently open in Fig. 12 stage. The ink-bag in Fig. 12 shows slightly irregular moniliform spaces, showing that its inward extremity presents its characteristic spiral twisting in this stage.

To complete the account of the digestive tract, another im-

portant organ, the "liver," has here to be dealt with. The "liver" arises as a paired structure from diverticula of the proctodæum. Figs. 5–8, Plate XVI, show a series of sections through the different regions of the "liver." Fig. 7 is the section passing through the region where the prolongation of the proctodæum sends out a pair of diverticula, one on each side of



the food yolk. The section that passes through somewhat above this region shows nothing but a stomach cavity and a part of the lateral diverticula. The section passing below the region figured in Fig. 7 shows two lobes of the liver as distinctly paired structures, and in a still lower plane the same paired structures are more widely separated, Figs. 6 and 5, *L*, *L*. The section,

Fig. 5, passes through the ink-bag situated just inside the rectum and surrounded by vascular spaces. Fig. 4 is the more magnified view of a section similar to Fig. 5. The section passes through the inward extremity of the ink-bag (*I*), and no lumen is seen in it.

Figure 13 shows a transverse section of a more advanced embryo through the region of the stomach and the "liver." The duct-like prolongation from the stomach *St.* to the glandular portion of the "liver" *L* is undoubtedly what has been described in the adult anatomy of the squid as the "spleen" *Sl.* The splanchnic ganglia, *Sp. g.*, is seen imbedded between the folds of the digestive tract. In no stage of the embryo does the "yolk membrane" take any share in the formation of the digestive tract. The lumen of the "yolk membrane" and that of the digestive tract remain entirely independent of each other. With the absorption of the food yolk the "yolk membrane" also vanishes, and the digestive function is assumed by the secondary set of digestive organs developed solely by the continuation of the stomodial and proctodial invaginations.

With this negative result to the inquiry on the fate of the "yolk membrane," whether it forms a part of the digestive tract or not, I think it is still possible to call the "yolk membrane" a true endoderm. Before entering, however, into this discussion it will be necessary to examine the more important results obtained by the preceding observers in regard to the origin of the germ layers of Cephalopods, and to compare them with those already described.

II.

As Köllicker's celebrated memoir ('**44**) on the development of various forms of Octopod and Decapod Cephalopods deals more especially with the external changes, I have little to do with it here in connection with the origin of the germ layers. It is necessary, however, to mention that he distinguishes two embryonic layers, the "innere" and the "äussere Schicht," which result from the splitting of the germ disk, and which, later, give rise to the inner and outer yolk sac and to all organs of the embryo. His statements that the digestive tract arises as a solid cord of cells which becomes hollowed out later, and that the "yolk membrane" is

continuous with the digestive tract, have been contradicted by some observers.

Metschnikoff ('67), whose account I take from a short German abstract, observed in Sepiola, at the third day of the first period, a division of the blastoderm into two lamellae which he calls "epitheliale" and "parenchymatose" respectively. From the former the body-wall, cartilage, sense organs, nutritive organs, with the exception of the pharynx and the ink-bag, develop, while from the latter the muscles, pharynx and vascular system take their origin. It is noticeable in his account of the origin of the digestive tract that he ascribes the major portion of it to the ectoderm.

Lankester's contributions ('73, '75*a*, '75*b*, '83) from time to time on the development of Cephalopods, extending for the length of ten years, require more extended notice here. His account of the early history of the germ layers may be best given in his own words, from the latest of his treatises on this subject ('83):

"In the Dibranchs the eggs are always very large; in some cases the amount of food yelk infused into the original egg-cell is so great as to give it the size of a large pea. This results in that mode of development which is only known outside this class among the Vertebrata; it is discoblastic. The protoplasm of the fertilized egg-cell segregates to one pole of the egg and there undergoes cell division, resulting in the formation of a disk of cleavage cells resembling the cicatricula of the hen's egg, which subsequently spread over and invest the whole egg. . . . . But in addition to the layer of cleavage cells, which consists of more than one stratum of cells in the future embryonic area as opposed to the yelk-sac area, additional cells are formed in the mass of residual yelk apparently by an independent process of segregation, each cell having a separate origin, whence they are termed 'autoplasts.' The autoplasts eventually form a layer of fusiform cells—the 'yelk membrane' which everywhere rests upon and encloses the residual yelk. The cleavage cells form a single layer on the yelk-sac area and two layers on the embryonic area, an outer one cell deep, and an inner—the middle layer of the three—which is often thick and many cells deep. There is a great difficulty here in identifying the layers with the three

typical layers of other animal embryos, except in regard to the outermost, which corresponds with the epiblast of Vertebrates in many respects. The middle layer, however, gives rise to the nerve ganglia as well as to the muscles, coelom, and skeleto-trophic tissues, and to the mid-portion of the alimentary canal with its hepatic diverticula, the liver. It is clearly, therefore, something more than the mesoblast of the Vertebrate, giving rise as it does to important organs formed both by epiblast and hypoblast in other animals. Lastly, the yelk membrane, though corresponding to the Vertebrate hypoblast in position and structure, furnishes no part of the alimentary tract, but disappears when the yelk is completely absorbed. In fact, the developmental phenomena in Sepia, Loligo, and Octopus are profoundly perturbed by the excessive proportion of food yelk. Balfour has shown that in the chick the orifice of closure of the overspreading blastoderm does not represent the whole of the blastopore, but that this is in part to be sought in the widely separated primitive streak. The present writer has little doubt that a structure corresponding to the primitive streak of the chick, and lying near the klastic pole, will be found in Sepia and Loligo, and the strange vesicular origin of the mid-gut will be traced to and explained by it."

His view of the "strange vesicular origin of the mid-gut" above-mentioned is based on the interpretation of a section similar to my Fig. 14, Plate XVII. This section is inconclusive, however, as other sections of the same specimen showed even at this stage that there is one point where the connection with the external ectoderm exists, although this continuity is not shown in the section from which Fig. 14 was drawn, nor in a similar section which Lankester figures. He says ('75*b*): "The epithelium of the chamber of the anal tubercle is in close contact, from the earliest stage at which I have seen it, with the epidermic epithelium, and much later at this point the anus breaks through. I have *not* found what might be looked for on *à priori* grounds (comparison with other molluscs), that the chamber of the anal tubercle is formed by an invagination from the external layer at this point." My sections, however, of Loligo and Grenacher's Cephalopod show this anal invagination conclusively, which Lankester expected to see on *à priori* ground. And the attempt to explain the "vesic-

ular origin" of that part of digestive tract in connection with the primitive streak-like structure in Cephalopod seems to me unnecessary; nor have I seen a structure anything like the primitive streak lying near the klastic pole, unless by it is meant the "pen sac" which shows in an early stage, an elongated shallow groove. His account of the autoplastic origin of the "yolk membrane" has been contradicted by subsequent observers. As to the homology of the "yolk membrane," he points out its strong resemblance to the vertebrate endoderm, but he does not assert that it is actually the endoderm. Speaking of this membrane he says ('75*b*), "Is this the hypoblast? Leaving aside speculative discussions, the question is, Does the epithelium of the middle portion of the alimentary canal develop from this layer?" Having satisfied himself, as I have also done, that the yolk membrane takes no part in formation of the permanent digestive tract, he concludes by pointing out its remarkable resemblance to the vertebrate endoderm, without committing himself to any view as to its homology.

By far the most detailed accounts of the early stages of the Cephalopods are found in the two memoirs of Ussow ('74*a*, '81). Ussow, working on the development of four different species of Cephalopods, namely, *Sepia*, *Sepiola*, *Loligo*, and *Argonauta*, at the same time, had an opportunity to compare the different forms, and his observations on the early stages of segmentation are of special value. However, his interpretation is open to confusion. He recognizes the existence of three embryonic layers, but of purely conventional kind and of no morphological significance. Thus he speaks of "obere Keimblatt" which corresponds to our ectoderm; the "mittelere Keimblatt" which he divides into two layers, "Hautmuskelschicht" and "Darmfaserschicht," corresponding to our mesoderm and the "yolk membrane" put together, but used in the same sense as applied to a higher vertebrate. To these two he adds a third, "Darmdrusenblatt," which is derived entirely from the invagination of the ectoderm, giving rise to the whole digestive canal and its appendages.

The "yolk membrane" then, according to him, is part of the mesoderm, and the morphological endoderm, as such, may be said to have no existence in Cephalopods.

Grenacher's ('74*b*) account of the formation of the digestive

tract in his interesting Cephalopod I have been able to confirm in my observations on the Cephalopod from Nassau. Like him, the collection at my disposal being scanty in the earlier stages, I have not been able to make out anything on the origin of the germ layers.

To Bobretzsky's original account ('**77**) I have no access. Balfour ('**80***b*) taking most of the illustrations of the development of Cephalopods from Bobretzsky's memoir, I may consider these two authors together. In regard to the "yolk membrane," Balfour says it is entirely passive and that it takes no share in the formation of the embryonic body; the mesoderm originates at the growing edge of the blastodisk, and that this mass of mesoblast contains endodermic elements, for later it gives rise to a part of the alimentary canal. It is evident that he sought the endoderm outside of the structure of the "yolk membrane." His account of the origin of the "mesenteron" is like that of Lankester, and he bases his statement on Bobretzsky's figure. He says that a band of mesoderm intervenes between the "mesenteron" and the ectoderm, thus giving an idea that there is no possible connection between the two. The figure above referred to corresponds to my Fig. 10, Plate XVII.

Brooks ('**80***a*), describing a stage in the developing embryo of the common squid, little older than my Fig. 3, Plate XVI, says: "The blastoderm now covers a considerable area at the formative pole of the egg, and its edge is marked by two parallel lines around the yolk. The outermost of these lines appears to be the growing edge of the layer of ectoderm which has been formed by the subdivision and increase of the segmentation spherules of an earlier stage, and the second or inner line I believe to be the growing edge of the lower layer of the blastoderm. The surface of the yolk below the blastoderm, and inside this second line, is covered by a number of large, well-defined, nucleus-like bodies, the autoplasts of Lankester, which are strictly confined to the area inside the inner one of the two circular lines noticed, and in no case reach to or beyond the growing edge of the blastoderm. The appearances indicate that these bodies are the nuclei of the lower layer, or endoderm, which is in process of formation on the surface of the yolk under the outer layer or ectoderm, but covering a smaller area, so that the ectoderm projects beyond the lower layer a little around the entire growing edge."

The observation here given on the living egg accords remarkably well with my sections, and the endodermic nature of the "yolk membrane" cells is distinctly pointed out.

To Paul Girod's exhaustive memoir ('82) on the ink-bag of Cephalopods allusion must here be made, inasmuch as he speaks of the development of the rectum and the ink-bag. Girod, as well as Ussow ('74*a*), describes the occurrence of the anal invagination as succeeding the elevation of the anal tract. According to them, first the anal area becomes elevated and forms a little eminence between the rudimentary gills on one side and a pair of siphon buds on the other. Upon this eminence a cavity is formed which, gradually working its way inward, differentiates into the ink-bag and the rectum. According to my observation, this elevation of the anal tract is not antecedent to the anal invagination, but concurrent with it. Because a certain group of ectodermic cells is carried underneath the general surface of the body, resulting in the formation of a spacious cavity and an accumulation of mesodermic cells around it, that region becomes more eminent than the surrounding. In the development of the anal area from the slightest elevation to a conspicuous eminence I observed a corresponding increase in the capacity of the underlying cavity or cavities.

Schimkewitsch ('86*a*), working on the development of *Sepia*, observed the formation of blood-corpuscles from the nuclei of the "yolk membrane" cells. Basing his fact on this, he carries the comparison of the "yolk membrane" to the "parablast" (His) of Teleosts. Although the comparison is a suggestive one, I have not been able to see, during my observations on Loligo, anything like the phenomena he figures and describes.

Bruce ('86*b*), basing his observations on the nature of the cells of the "yolk membrane" and their nuclei, recognized these cells as the endoderm. "These cells," he says, "from their spindle shape and oval nuclei, are readily recognized as the endoderm cells which form the inner coating of the yolk sac and digestive tract in more advanced embryos."

Vialleton ('87), working on the development of the squid, describes in the early stage of segmentation the existence of two elements which he calls respectively "blastomères" and "blastocones." The former corresponds to our central and intermediate

zone put together, where the segments are more or less individualized, and the latter to our marginal zone, where protoplasm becomes thinner and the outline of each segment is indistinct. From the "blastomères" the body of the embryo is developed, while the "blastocones" form a plasmodium and give rise to the perivitelline membrane ("yolk membrane"). The cells of the "blastomères" increasing very rapidly necessarily encroach upon the "blastocones," which directly rest upon the food yolk, until finally the cells of the "blastocones" become intercalated between the "blastomères" and the food yolk. Later the perivitelline membrane entirely separates the "blastomères" from the yolk. He regards the behavior of the perivitelline membrane as one mode of formation of the endoderm.

In regard to the origin of the "yolk-membrane" cells in the marginal zone, my observation agrees with his description. But as to the cause of intercalation due to the rapid multiplication of his "blastomères" resulting in the encroachment upon the surface of the "blastocones," my sections do not admit this mode of interpretation. I am more inclined to believe the "yolk-membrane" cells originate by the direct division of the lower stratum of protoplasm in the marginal zone of the disk, or possibly by the direct migration of marginal zone cells in different points, but not as a result of the mechanical encroachment of the rapidly increasing inner area or the "blastomères."

III.

It will be seen from the foregoing that the range of opinions in regard to the nature of the "yolk membrane" and the origin of the digestive tract is quite extensive.

With the exception of Lankester, all agree in describing the "yolk membrane" as originating from the segmentation of the original germ disk. I do not clearly understand what views as to the homology of the "yolk membrane" are held by Köllicker and Metschnikoff; Lankester and Balfour do not definitely commit themselves to any homology with the germ layers of other animals. Ussow calls the "yolk membrane" a part of the mesoderm, while Brooks, Schimkewitsch, Bruce and Vialleton point out more or less distinctly its endodermic nature.

As to the origin of the digestive tract, opinions are equally

diverse. Köllicker and Bruce describe the continuity of the digestive tract with the "yolk membrane." Balfour and Lankester describe the "mesenteron" as arising from the lower layer of the mesoderm. Metschnikoff derives the major part of it from the ectoderm, while Ussow attributes the formation of the entire tract of the digestive canal to the ectodermic involutions.

In regard to these disputed points I have satisfied myself in observing the following: (1) The "yolk membrane" is derived from the original germ disk; (2) It makes its appearance underneath the marginal zone of the germ disk near to the junction of the intermediate zone; (3) The cells of the "yolk membrane" are irregular in outline, fusiform in cross section, amoeboid in movement, each with one or more large nuclei; (4) With the growing edge of the blastoderm the underlying "yolk membrane" keeps pace in growth with the spreading germ disk, but never coming outside of the peripheral zone; (5) the "yolk membrane" grows at the same time from the margin to the centre of the disk, and in time it comes to completely separate the blastodisk from the yolk mass; (6) The blastodisk at about this stage is a hollow hemisphere placed at one pole of the yolk mass, and with the origin of the "yolk membrane" cells underneath the marginal zone, which grow centripetally underneath the blastodisk, presents a phenomenon comparable in all respects to the epibolic gastrula; (7) Hence I necessarily believe the "yolk membrane" to be a true endoderm and its sole representative in the Cephalopods. (8) As to the origin of the digestive tract with its appendages, I found it entirely to be formed by the ectodermic invaginations, that is, by the prolongations of the proctodæum and the stomodæum; (9) At no period of the development does there exist any connection between the "yolk membrane" and the digestive tract, and long before the absorption of the food yolk is completed the permanent digestive canal is formed; (10) With the absorption of the food yolk the "yolk membrane" disappears.

As to the significance of the extraordinary origin of the definitive digestive tract in exclusion of the "yolk membrane" or true endoderm, the presence of an enormous food yolk seems to account for it, as I have pointed out in the "University Circulars" ('88). This extraordinary peculiarity is the most distinctive characteristic of Cephalopod embryology. The food yolk attains such an

enormous bulk in the Cephalopod that the relation which it bears to the germinal disk is said to reach its maximum development in the whole Invertebrates. The amount of the food yolk is so great that long after the embryo attains the full morphological characters of the adult, the food yolk is still seen stored inside of the body; and no doubt to this fact of ready and abundant nutrition during the embryonal period is due, I believe, the almost total absence of metamorphosis in the development of Cephalopods. In other words, owing to the large amount of the food yolk to be digested, other morphological structures, as the entire tract of the digestive canal with its appendages, are completed before the absorption of the food yolk has taken place, and the share the "yolk membrame," which is morphologically a mid-gut, would otherwise contribute in the formation of the digestive canal is thus left out. What Ryder[1] observes on the development of Vertebrates applies with equal force in the development of molluscan series, when he says: "With the gradual increase in the size of the yelk in the vertebrate series, it finally happens that the principal morphological features of the embryo are distinctly developed long before yelk absorption is completed. As a result of this, the yelk, which occupies a ventral position in reference to the intestine, is finally excluded from direct connection with the latter, and the periblast (hypoblastic in origin) is made to assume a new function, viz. that of ministering to the incorporation of the vitelline matter."

Substitute "molluscan series" to "vertebrate series" and "yolk membrane" to "periblast," and the parallelism between the developmental features of the two groups in this respect is complete. The endoderm in the highest group of the molluscan series, the Cephalopoda, is made a temporary embryonic structure which may be said to have no chance of leaving traces of definite structure in the organization of the adult, and the set of secondary digestive organs in addition to the primary one is developed from entirely different source, the ectoderm.

Observing then that a certain portion (the mesenteron) of the digestive tract of the several forms of Mollusca is derived from the original endoderm, and further, that the "yolk membrane" in

[1] "On the Development of Osseous Fishes." John A. Ryder. Report of the Commissioner of U. S. Fish and Fisheries for 1885.

Cephalopods is the homologue of the other molluscan endoderm, the two structures, however, must by no means be taken to be identical in the history they pursue. In the Cephalopod the endoderm becomes the yolk digesting membrane, and before it can dispense with this function, the prolongations of the fore and hind gut complete the digestive tube and exclude the endoderm, which itself becomes absorbed later. In some other Mollusca the endoderm becomes the permanent mesenteron and persists throughout the life of the animal. In other words, the digestive organs of the adult animals in the group Mollusca are not of similar origin, or, more concretely, the digestive systems of the squid and snail are not homologous. This may possibly be an extreme instance of the influence which the food yolk exerts in modifying the course of development and the history of the germ layers.

BIOLOGICAL LABORATORY, JOHNS HOPKINS UNIVERSITY, March 30, 1888.

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EXPLANATION OF FIGURES, PLATES XVI AND XVII.

LIST OF REFERENCE LETTERS.

A. Anus.
Ad. s. Retractor muscle of the siphon.
Bl. Blastopore.
Ep. Ectoderm.
F. Y. Food yolk.
G. Gill.
Hy. Endoderm ("yolk membrane").
I. Ink-bag.
L. "Liver."
Ms. Mesoderm.
Mt. Mantel.
Mt. c. Mantel cavity.
Oe. Oesophagus.
R, R.' Rectum.
Sl. "Spleen."
Sp. g. Splanchnic ganglia.
St. Stomach.
St. g. Stellate ganglia.
Y. M. "Yolk membrane" (endoderm).

Figs. 5, 6, 7, and 8 are drawn with Zeiss' Obj. A and Oberhausen camera; all the other figures are drawn with Zeiss' Obj. D and Oberhausen camera. All have been reduced to one half the size of the original drawings.

PLATE XVI.

FIG. 1.—(Loligo Pealii). Vertical section through the blastodisk of a young ovum.

FIG. 2.—(Loligo Pealii). The same, of a more advanced ovum. The germ disk is differentiated into three concentric zones: a central, one cell thick; the intermediate, two cells thick; and the marginal, single cell thick zone. The spindle-shaped endoderm (*Hy.*) cells are seen intervening between the germ disk and the food yolk. The endoderm cells do not yet extend completely underneath the germ disk.

FIG. 3.—(Loligo Pealii). The same, still more advanced; the endoderm completely separates the germ disk from the yolk mass.

FIG. 4.—(Loligo Pealii). Transverse section of an advanced embryo, already hatched, showing the relative position of the rectum (*R*), ink-bag (*I*), "liver" (*L*), etc. The food yolk remains still unabsorbed inside the body, completely enveloped by the endoderm or the "yolk membrane" (*Y. M.*).

FIGS. 5–8.—(Loligo Pealii). Transverse sections of the advanced embryo 3 mm. in length, showing the position of the "liver" in different planes of the body. In Figs. 5 and 6 the "liver" (*L*) is seen as a paired structure on both sides of the food yolk (*F. Y.*). In Fig. 7 the "liver" becomes continuous with the prolongations of the proctodial cavity (*St.*); in Fig. 8 only a portion of the hepatic diverticula is seen by the side of the cavity (*St.*).

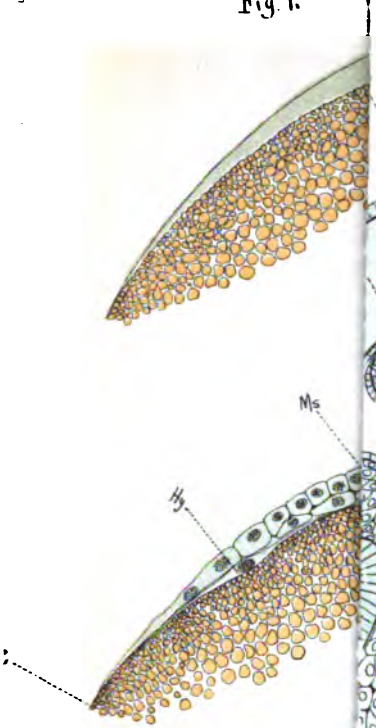
PLATE XVII.

FIG. 9.—(Loligo Pealii). Longitudinal section of a young, unhatched embryo, showing the invagination of the ectoderm, resulting in the formation of the ink-bag (*I*) and the rectum (*R'*). Cells lining the cavity of the ink-bag are vacuolated and contain the mucus-like fluid.

FIG. 10.—(Loligo Pealii). Longitudinal section of a young embryo of the same stage as Fig. 9. The plane of the section passes little beyond the point of invagination. Rectal cavity (*R'*) completely separated from the exterior by a group of mesoblast cells (*Ms.*).

FIG. 11.—(Loligo Pealii). Longitudinal section of a young embryo,

Fig. 1.



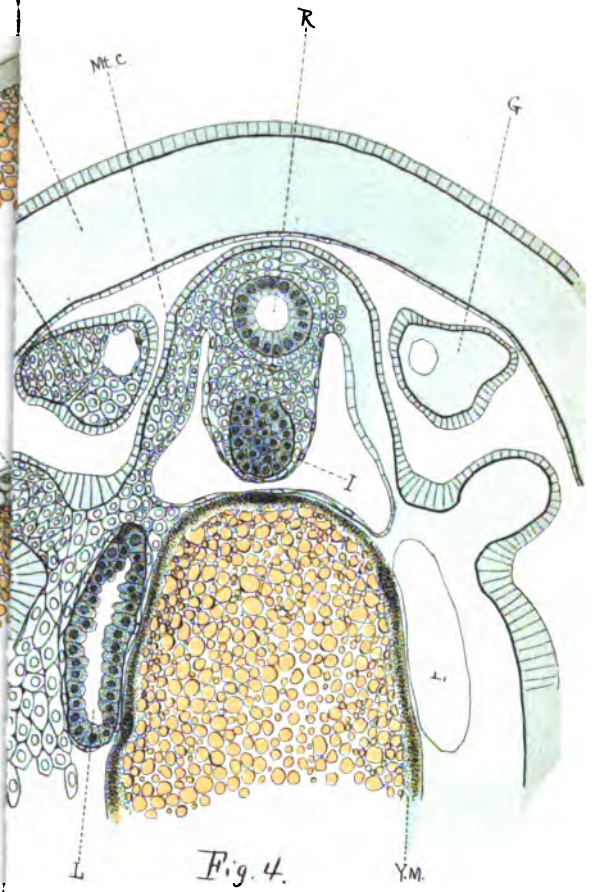


Fig. 4.

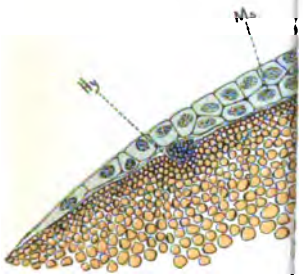


Fig. 7.

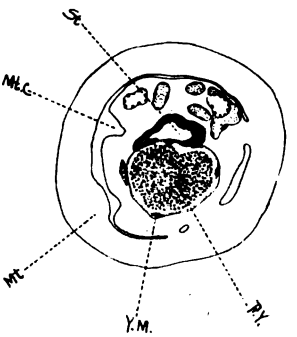


Fig. 8.

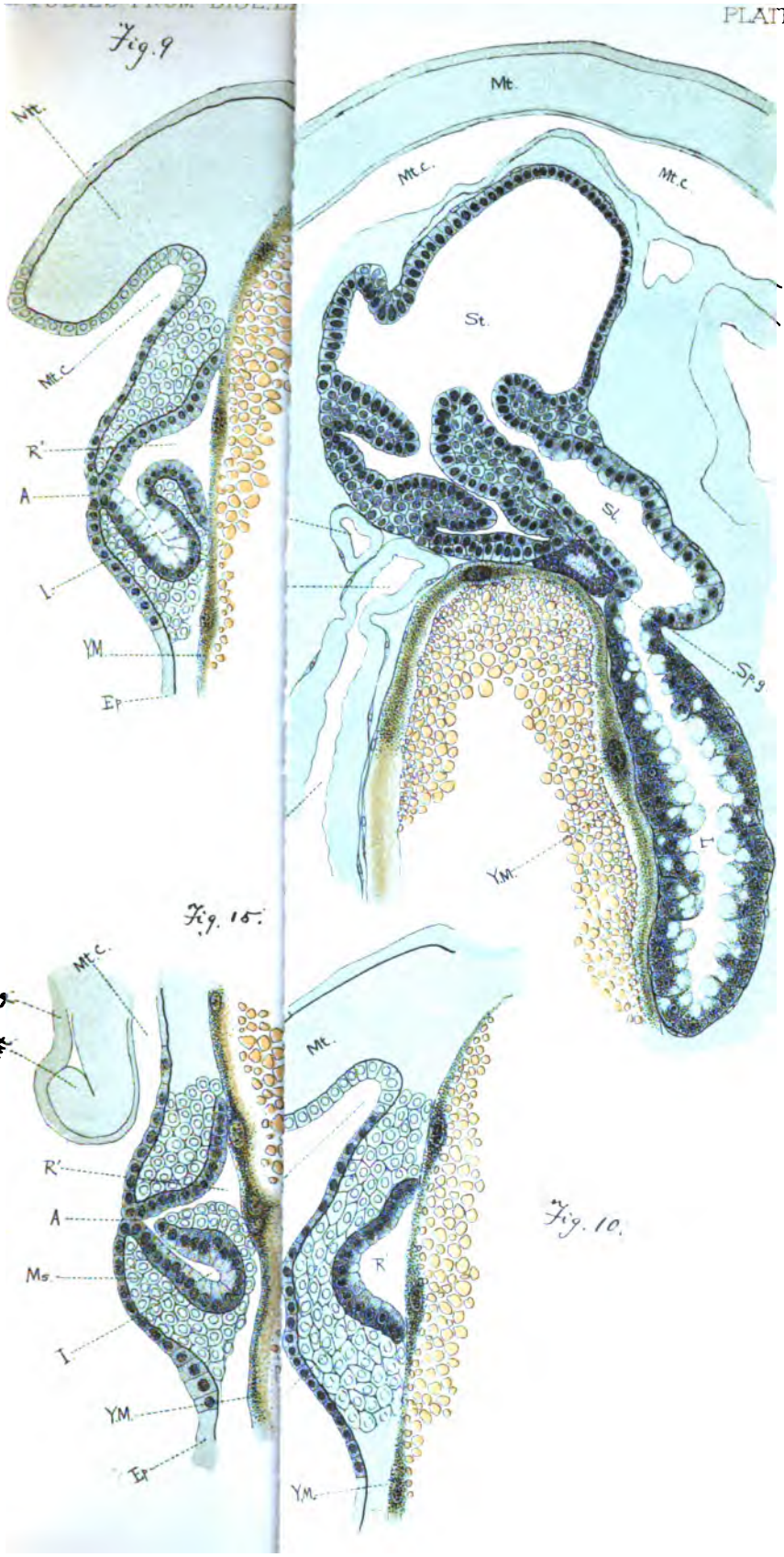


Fig. 9
Mt.
Mt.c.
R'
A
I
YM.
Ep
Mt.
Mt.c.
Mt.c.
St.
Sl.
Sp.g
I
YM.
Fig. 15.
Mt.c.
Ep
Mt.
R'
A
Ms.
I
YM.
Ep
Mt.
R
YM.
Fig. 10.

unhatched, but more advanced than Figs. 9 and 10 stage. The ink-bag (*I*) and the rectum (*R*) well differentiated. The mesoblast around the ink-bag differentiate themselves into a well defined layer. The roof of the rectum (*R*) travels dorsalward over the endoderm or the "yolk membrane" (*Y. M.*).

FIG. 12.—(Loligo Pealii). Longitudinal section of a still more advanced embryo through the point of anal orifice (*A*), showing the further extension of the rectum (*R*) dorsalward, forming a spacious cavity, the stomach (*St.*), which is continuous with prolongation of the stomodæum or the oesophagus (*Oe.*). The ink-bag (*I*) shows the moniliform lumen which is due to the slight spiral twisting of the bag toward its inward extremity.

FIG. 13.—(Loligo Pealii). Transverse section of a well advanced embryo (4 mm. in length) through the region of the stomach (*St.*) and "liver" (*L*), corresponding to the section Fig. 7, but in more advanced stage. Histological differentiation in the cells lining the stomach (*St.*), the "spleen" (*Sl.*) and the "liver" (*L*) are well marked. The splanchnic ganglia (*Sp. g.*) is found imbedded in the folds of the digestive tract. The food yolk still exists in a large quantity, completely enveloped by the endodermic membrane (*Y. M.*).

FIG. 14.—(Grenacher's Cephalopod from Nassau.) Longitudinal section of a young specimen about 3 mm. in length, through the region of the anal eminence. The section does not pass exactly through the point of ectodermic involution, but little beyond it. The manner of formation of the ink-bag (*I*) and the rectum (*R'*) is essentially the same as in the squid.

FIG. 15.—(Grenacher's Cephalopod from Nassau.) This is from the same specimen from which the section Fig. 14 is taken. The plane of section passes through the point of involution, showing the continuity of the ectoderm to the walls of the rectum (*R'*) and the ink-bag (*I*).

DEVELOPMENT OF THE EUSTACHIAN TUBE, MIDDLE EAR, TYMPANIC MEMBRANE AND MEATUS OF THE CHICK.

By F. MALL, M. D., Fellow in Pathology, Johns Hopkins University, Baltimore.

With Plates XVIII and XIX.

Formerly the Eustachian tube, middle ear and meatus were described as arising directly from the first branchial cleft. The description was very simple, it being that the first and second branchial arches united by the growing together of the epithelium, and that the union thus formed became converted into the tympanic membrane. The depression external[1] to this union was changed into the meatus, and the depression internal to the union was converted into the Eustachian tube and the tympanic cavity.

Hunt[2] denied this statement and asserted that "the Eustachian tube is an involution of the pharyngeal mucous membrane; the meatus is an involution of the integument; the drum is formed by the Eustachian tube overlapping the extremity of the meatus." His investigations were upon the embryo pig.

A year later, Moldenhauer[3] carefully studied the subject in question upon the chick, and he also differed from the older teachings. He described a ridge in the pharynx (colliculus tubo-pharyngeus) which extends from the first cleft nearly to Rathke's pocket. Anterior to this ridge is a sulcus (sulcus lingualis), and posterior to it another groove, beginning at the inner opening of the first branchial cleft and gradually becoming shallower and shallower as it extends upwards. This groove he calls sulcus tubo-tympanicus, and from it he believes the Eustachian tube and tympanic cavity arise. The external ear passage is formed by tissue being built around the posterior part of the first cleft which has by this time become closed; the uniting membrane produced by the union of the first and second arches is now opposite the canalis tubo-tympanicus, and in this way the tympanic membrane is formed.

[1] I shall use synonymously: upper and oral, lower and aboral, anterior and ventral, and posterior and dorsal.

[2] Transactions of the International Otological Congress of 1876.

[3] Morpholog. Jahrbuch, Bd. III, 1877.

Urbantschitsch[1] fully agrees with Hunt, stating also that the first cleft has nothing at all to do with the tube, tympanic membrane, or meatus. According to him the tube is an involution of the endoderm, the meatus an involution of the ectoderm, both being within the tissue of the first arch. The tympanic membrane is formed by the overlapping of the ectodermal and endodermal involutions.

Kölliker[2] states that the inner and outer parts of the ventral half of the first and second arches unite, and at the dorsal part, the primary point of closure is converted into the tympanic membrane. The Eustachian tube, according to Kölliker, is undoubtedly developed from the posterior part of the median half of the first branchial cleft. The middle ear is developed by a new outgrowth from the outer part of the tube, the outgrowth (canalis tubo-tympanicus, Kölliker, sulcus tubo-tympanicus, Moldenhauer) extending outwards, upwards and backwards.

Allen Thomson[3] retains the old teachings. He cannot accept the views of Hunt, Moldenhauer, or Urbantschitsch because they do not give sufficient evidence upon which to base their views.

Hoffman[4] examined the embryos of reptiles, birds and mammals, his investigations leading him to agree with Kölliker as regards the development of the tube and middle ear, though differing from him regarding the direction of the growth of the canalis tubo-tympanicus.

Lately Kastschenko[5] and Gradenigo[6] have investigated the development of the auditory apparatus, and both come to the conclusion that it is not derived directly from the first branchial cleft. Kastschenko finds that the middle ear is formed by the narrowing of the lateral walls of the pharynx in the region of the second branchial arch. The tympanic membrane is formed mostly from the tissue of the second arch.

The branchial arches[7] of the chick appear at the end of the

[1] Mittheil. aus d. Embryolog. Institut. Wien, 1877.

[2] Grundriss d. Entwicklungsgeschichte d. Menschen. 1884.

[3] Quain's Anatomy, 9th edition, p. 854.

[4] Arch. f. Mik. Anat., Bd. 23.

[5] Ibid. Bd. 30, 1887.

[6] Centralblatt f. Med. Wiss., 1886, and Mittheil. aus d. Embryolog. Institut, Wien, 1888.

[7] Mall. Arch. f. Anat. u. Phys., Anat. Abt., 1887. Fig. 1 in this article is a copy of Fig. 3, Plate I, of that article.

second day. The head now rapidly flexes upon the body, and at the end of the third day the arches are fully formed. From now on the head gradually extends itself, so that at the end of the fourth day the head is bent upon the body at about a right angle (Fig. 1). In this figure the first external branchial groove is tadpole-shaped. On the aboral border of the first arch and on the oral border of the second arch are the colliculi branchiales externi of Moldenhauer. Between these four tubercles the meatus is formed. Posterior to the colliculi bran. ex. is a "pin-point" depression which is an invagination of ectoderm uniting with the ganglion of the facial nerve.[1] The lower part of the second arch is beginning to separate from its upper half in order to cover the third and fourth arches. During the following day the ear is nearly developed, and the pin-point depression posterior to it has disappeared. At the end of the sixth day the external ear is fully formed.

In 1881 His[2] demonstrated that the branchial clefts are not fissures, but that ectoderm and endoderm dip into the cleft, meet each other and thus form a closing membrane (*Verschlussplatte*). He made his observation on the human embryo, and since then it has been verified in the embryo of the pig,[3] of the rabbit[4] and in the chick.[5]

Fol and lately his pupil, de Meuron,[6] deny the existence of this "membrane of His" for all classes of animals, while Froriep[7] mentions that in a cow's embryo the clefts were not fully closed. Froriep is cautious enough not to state definitely whether or not the open cleft was due to the preparation of the material.

Kastschenko[8] states very definitely that in birds the second and third "clefts" are true clefts, each having a dorsal (*Schlundloch*) and a ventral (*eigentliche Schlundspalte*) opening; the first

[1] Froriep. Arch. f. Anat. u. Phys., Anat. Abt., 1885.

[2] His. Arch. f. Anat. u. Phys., Anat. Abt., 1881.

[3] Born. Arch. f. Mik. Anat., 1883. Fischelis. Ib. 1885. Kastschenko. Ib. 1887.

[4] Kölliker. loc. cit.

[5] Kölliker. loc. cit. Mall. Arch. f. Anat. u. Phys., Anat. Abt., 1887.

[6] De Meuron. Recherches sur le développement du Thymus, etc. Inaug. Diss. Genève, 1886.

[7] Froriep. Arch. f. Anat. u. Phys., Anat. Abt., 1885.

[8] Kastschenko. Arch. f. Anat. u. Phys., Anat. Abt., 1887.

cleft has only the dorsal (*Schlundloch*) opening. Also Leissner[1] states that as a rule the first three clefts are true clefts.

It is easy to understand Kastschenko by glancing at his beautiful reconstructions. His dorsal openings (*Schlundloch*) of the clefts are, I believe, the "branchial sense organs" of Froriep and of Beard.[2] The sense organs of the second and third clefts never appeared to me as openings, but the organ of the first cleft caused me considerable trouble. I finally succeeded in tracing the ectoderm throughout the invagination to the seventh nerve, as shown in Figs. 2, 3 and 4. Regarding the ventral openings of the second and third clefts I can only say that during existence of the third cleft I have never seen it open. In sections of chicks of 88 hours and 99 hours occasionally a section or two appears in which the second cleft on one side is open; and then generally can the point of the tearing of the "closing membrane" easily be recognized. The fact that this broken "membrane of His" was present on one side only and then in but a section or two at a time, easily accounts for Leissner finding many small openings in a chick of 90 hours.

I look upon these openings in the membrane of the second cleft as artificial, for they are not symmetrical, and nearly always can the point of rupture be detected. Besides, if these openings were not artificial it would be extremely difficult to account for the continuous ectoderm and endoderm as I find it in a chick of 112 hours.[3] Finally, the convincing argument to me is that in a series of sections of dog's embryos,[4] at no time were the clefts found open.

Since we have the "membrane of His," the difficulty of the formation of the tympanic membrane is easily solved. We now have from the beginning an external and an internal part to the cleft, and all we need is mesoderm between the two layers of the "membrane of His" and the tympanic membrane is complete.

In examining Fig. 2, which is a reconstructed section between the first and second arches so as to cut the "membrane of His" throughout its whole extent, it is seen that the hinder brain, chorda, jugular vein and bulbus aortae are cut across. The

[1] Leissner. Morphologisches Jahrbuch, Bd. 13, 1888.
[2] Beard. Studies from Biolog. Laboratory, Owens College, Vol. I.
[3] Mall. Arch. f. Anat. u. Phys., Anat. Abt., 1887, Plate II, Fig. 27, II.
[4] The article following this.

internal branchial pocket[1] is well formed and extends to the ectoderm. The external branchial groove extends around the ventral half of the neck, is deep anteriorly and becomes shallower as it passes posteriorly. Laterally to the internal pocket there is the beginning of an involution (VII) of ectoderm which comes to meet and is to overlap the involution from the endoderm.

Figure 3 is a reconstructed section through the first branchial cleft of a chick of two days and twenty-two hours. We see that the first arch is fully formed and that the pharynx has become absolutely narrower than formerly, while its dorso-ventral diameter has increased. The internal pocket is wider, deeper and longer than before, and its oral and aboral walls lie in apposition to each other. The ectodermal involution (VIII) has now extended half way to the median line and lies dorsalwards from the internal pocket.

The same reconstruction from a chick of three days and sixteen hours (Fig. 4) gives almost the same picture. The pharynx has become wider than before, the first branchial pocket has been prolonged, retains its former position, and, instead of having a rounded outer extremity, is pointed. The ectodermal involution (VII) is deeper than formerly, and, on account of the growth of the body, now does not reach more than one third the distance to the median line. Ventralwards from the ectodermal involution (VII) is a second depression (*m. e.*), which is the beginning of the external ear passage. If we compare this latter reconstruction with Fig. 1 we see how well they coincide; how the ectodermal involution (Fig. 4, VII) corresponds to the "pin point" depression (Fig. 1, VII) at the posterior part of the first external branchial groove; how the second involution (Fig. 4, *m. e.*) corresponds to the depression (Fig. 1, *c. br. ex.*) surrounded by the colliculi branchiales externi.

In the embryo of Fig. 3 the facial ganglion is just dorsal to the ectodermal involution, while in the embryo of Fig. 4 the ganglion is absolutely blended with the pin-pointed depression (Fig. 5).

When we pass from the embryo of three days and sixteen hours to one of five days we notice that no material change has taken

[1] The depression external to the "membrane of His" is groove-like, therefore I shall speak of it as branchial groove. The depression internal to the membrane is sack-like, therefore branchial pocket. The pocket and the groove form the "cleft."

place, except that about on this day a sack-like prolongation is sent from the extreme outer part of the first internal pocket, and extends upwards, slightly outwards and *forwards* (Fig. 6). This prolongation has been called by Kölliker, canalis tubo-tympanicus. During or possibly before the canalis tubo-tympanicus is formed, the ectodermal involution posterior to the colliculi branchiales externi is, with the exception of its inner part, which blends with the facial nerve, retracted. It seems, therefore, as if the "processus" tubo-tympanicus were to cut off this involution.

In an embryo of five days and sixteen hours (Fig. 7) the canalis tubo-tympanicus is wider and communicates more freely with the internal pocket (now better called Eustachian tube) than it did in the embryo of five days. The mesodermal tissue between the canalis tubo-tympanicus and outer end of the first internal branchial pocket on one side, and the depression between the colliculi branchiales externi on the other side, has increased in quantity.

Until this stage is reached the head is quite well flexed upon the body, and the direction of the first internal branchial pocket, with its prolonged outer extremity (canalis t. t.) is outwards, forwards and upwards. Through the erection of the head and the formation of the neck the Eustachian tube and middle ear are turned around an axis which is nearly parallel with the Eustachian tube. After the head is erected the direction of the canalis tubo-tympanicus is upwards, outwards and *backwards*, and the tube opens into the lower anterior angle of the tympanic cavity.

Figure 8 is a reconstruction of the auditory apparatus of an embryo of ten days. The figure shows that the meatus and the tube are in a straight line. The sections showed that the direction of the tube was slightly upwards, directly outwards, and backwards at an angle of 30°. The reconstruction cannot, of course, show this angle. The tube opens into the anterior and lower part of the drum cavity. The meatus is very deep and meets the tube at a very obtuse angle. The tympanic membrane is fully formed, and in it has become imbedded one end of the columella which extends obliquely upwards across the middle ear, but at no point fully separated from the drum cavity wall, and ends in the fenestra ovalis.

Résumé.

The first internal branchial pocket is converted into the Eustachian tube. The meatus is formed principally by the colliculi branchiales externi forming a wall around the posterior part of the first external groove. The "membrane of His" receives mesoblastic tissue between its two layers, and thus forms the tympanic membrane.

During the third day of incubation an ectodermal involution is formed from the dorsal part of the first external branchial groove. This involution lies in direct apposition with the dorsal part of the first internal branchial pocket and blends with the facial nerve. During the fifth day of incubation the connection between the facial nerve and the ectoderm is severed, and a new outgrowth (canalis tubo-tympanicus) from the outer part of the first internal branchial pocket takes its place. This new outgrowth first extends outwards, upwards and forwards, but through the erection of the head its direction is changed to outwards, upwards and backwards. It forms the tympanic cavity.

In conclusion, I take this occasion to express my obligations to Professor His, of Leipzig, in whose laboratory this work was performed.

DESCRIPTION OF PLATES XVIII AND XIX.

FIGURE 1.—Embryo of 4 days. × 10 diameters. I, II, III and IV, branchial arches. VII, "pin point" depression at the dorsal part of the first cleft. *c. br. ex.*, colliculi branchiales externi.

FIG. 2.—Reconstructed section through the first branchial cleft of a chick 2 days and 6 hours. × 40 diameters. *M*, medullary canal. *Ph.*, pharynx. *B. a.*, bulbus aortae. *v. j.*, jugular vein. *brp'*, first internal branchial pocket. *ec.*, ectodermal layer of the membrane of His cut across. VII, "pin point" depression at the posterior part of the first external groove. *Br'*, first branchial arch.

FIG. 3.—Reconstructed section through the first branchial cleft of a chick 2 days and 22 hours. × 40 diameters. *M*, medullary canal. *Ph.*, pharynx. *brp'*, first branchial pocket. *Br'*, first branchial arch. *ec.*, ectodermal layer of the membrane of His. VII, "pin point" depression at the posterior part of the first external groove.

FIG. 4.—Reconstructed section through the first branchial cleft of a chick 3 days and 16 hours. × 40 diameters. *M*, medullary canal.

a. v., auditory vesicle. *Ph.*, pharynx. *brp'*, first internal branchial pocket. *Br'*, first branchial arch. VII, pin point depression at the posterior part of the first branchial groove. *m. e.*, beginning of the meatus. *ec.*, ectoderm.

FIG. 5.—Section through the "pin point" depression of the embryo of 3 days, 16 hours. × 225 diameters. *ec.*, ectoderm. *Brf'*, the "pin point" depression as it passes in and unites with the facial nerve, N^{VII}.

FIG. 6.—Section through the first cleft of an embryo of 5 days. × 160 diameters. *ec.*, ectoderm. *brp'*, first internal branchial pocket. *c. t. t.*, canalis tubo-tympanicus. N^{VII}, 7th nerve.

FIG. 7.—Section through the first branchial cleft of an embryo of 5 days, 16 hours. × 60 diameters. *M*, median line. VII, 7th nerve. *Ph.*, pharynx. *c. t. t.*, canalis tubo-tympanicus. *ec.*, ectoderm. *m. ex.*, meatus. The union with the 7th nerve is shown in a few sections more dorsalwards. The meatus is struck squarely $\frac{1}{100}$ of a millimeter more ventralwards.

FIG. 8.—Reconstructed section through the auditory apparatus of a chick 10 days. × 20 diameters. *M*, median line. *Ph.*, pharynx. *Cav. T.*, drum cavity. *Ty.*, tympanic membrane. *meat. ex.*, meatus. *Col.*, columella. *F. O.*, fenestra ovalis. *L.*, labyrinth.

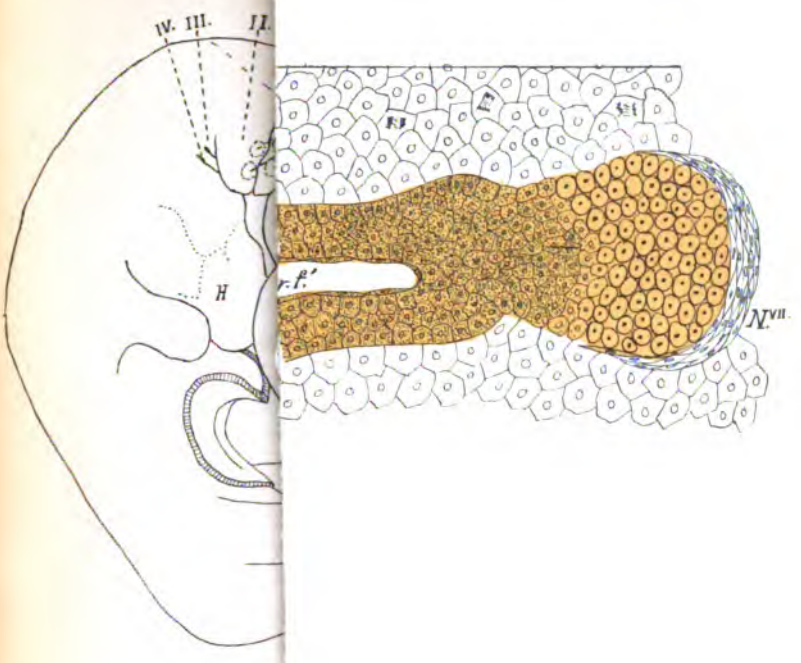


FIG. 8.

F1

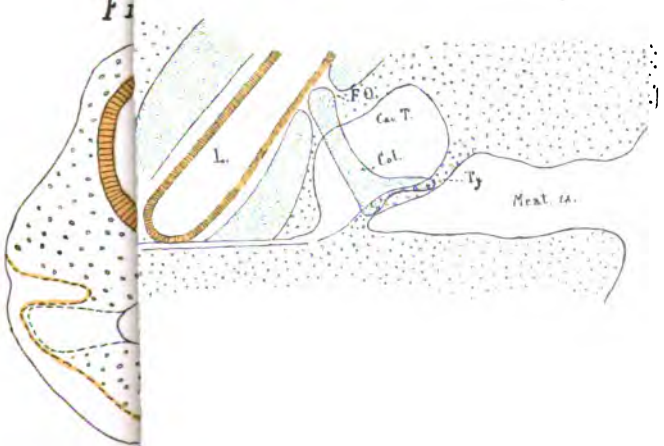


PHOTO LITH. BY A. HOEN & CO. BALTIMORE, MD.

THE BRANCHIAL CLEFTS OF THE DOG, WITH SPECIAL REFERENCE TO THE ORIGIN OF THE THYMUS GLAND. With Plates XIX, XX and XXI. By F. MALL.

During the year 1885 I studied, under the direction of Prof. His, the branchial region of the chick. The investigation included the derivatives of the clefts, and as I was seeking the origin of glandular organs only, the nerves were omitted. In that work I most definitely came to the conclusion that the thymus arises from the ectoderm; a result which is in absolute contradiction to Prof. His's result obtained from the human embryo. In a paper[1] which appeared before the publication of mine,[2] Prof. His brings forth new facts to prove that his former view regarding the origin of the thymus is correct. This has induced me to rework the whole subject in question on some mammalian embryo, and since a collection of dog embryos is in my possession, I have utilized them.

After microtoming the embryos I thought that a short paper would suffice, but while working the sections into reconstructions I found it necessary to include all the arches and clefts, and also the nerve ganglia of the branchial region.

The embryos were hardened by opening the cut-out uterus under a 10 per cent solution of nitric acid. This immediately coagulates the tissues without causing any irregular shrinking. The embryos were then preserved in 80 per cent alcohol. From the collection four stages were selected, carefully drawn, stained with haematoxylin and eosin, imbedded in paraffine, and cut on a Schanze microtome into sections .02 mm. thick. During the staining and imbedding all the embryos were kept side by side. The direction of the section was carefully drawn upon the original drawing, and great pains were taken to get the section through

[1] Ueber den Sinus praecervicalis u. Thymus. Arch. f. Anat. u. Phys., Anat. Abt., 1886.

[2] Entwicklung d. Branchialbogen u. -spalten des Hühnchens. Arch. f. Anat. u. Phys., Anat. Abt., 1887.

the branchial region accurately transverse. The sections proved to be a perfect series; nicely stained, none distorted, and none lost.

The dorsal reconstructions were made according to His's method, and the profile reconstructions according to Froriep's modification. The original drawing was used as a basis into which the sections were replaced as you push the drawers into a bureau. The original drawings were made from the embryos while in alcohol. The paraffine imbedding method causes the embryo to shrink, and, therefore, if the original drawing is enlarged to 50 diameters it must be reduced so that the drawings of the sections enlarged 50 times will fit accurately into it. This reduction caused me considerable trouble, for I found that the percentage of shrinkage (given in the table) is not the same for all embryos. When the scale of the original drawing and that of the section are the same, and when the direction of the section is accurately made out, the original drawing needs only to be ruled and each section will fit into its place.

	Length.				Percentage of Shrinkage	
Embryo.	Nape-breech.[1]	Vertex-breech.	In HNO_3 (10 per cent.)	In Alcohol (80 per cent.)	while Imbedding.	Eosin Staining.
A	6 mm.	5½ mm.	½ hr.	2 mo.	28.5%	Excellent.
B	8½	5	2	11	11	None.
C	10	9	18	1	2	Good.
D	13½	15	6	12	14.7	Fair.

Embryo A.

The embryo has the lower part of the body bent to one side, and, therefore, the length of the embryo cannot be made out definitely. The picture straightened gives a nape-breech length of 6 mm. and a vertex-breech length of 5½ mm. The head is turned to one side and well flexed upon the body, while the lower part of the body is already well formed.

The primary ocular vesicle is beginning to be invaginated, and the ectoderm which is later to form the lens is already thickened. Directly dorsalwards[2] from the base of the second branchial arch

[1] I shall use the terms "nape-breech" and "vertex-breech" for the German "Nackenlinie" (His. Anat. Mensch. Em., Th. I u. II) and "Steiss-Scheitellinie" (Born. Arch. f. Mik. Anat., Bd. 22).

[2] Whenever necessary, to avoid ambiguity, I shall use the terms oral-, aboral-, dorsal-, ventral-, median-, and lateralwards. Also, up as oral; down as aboral; anterior as ventral; and posterior as dorsal.

lies the auditory vesicle, which is fully separated from the ectoderm (Fig. 1). Upon the Wolffian ridge there is the beginning of the anterior extremity. In general, the embryo is about as far advanced as a chick of three days.

The branchial arches are all present, their relative size being shown in Fig. 1. From the first arch the superior maxillary process arises and extends, at right angles from the body of the arch, over the embryonic eye.

The clefts[1] are all well marked, especially at their dorsal parts, where they end in large, deep depressions. These depressions correspond with the "pin-point" depressions which I have already mentioned[2] as being present in the chick. The first and second branchial grooves are about of the same shape, both being tadpole-shaped, while the third and fourth grooves show something very remarkable. Viewed with a weak lens, no fourth arch can be seen, but in its place there is an oval depression, in the ventral part of which there is a small mound. A careful reconstruction shows (Fig. 1) that the mound is the fourth arch, and the depression dorsalward from it is a common depression for both third and fourth branchial furrows.

The relative position of the descending aorta, carotid artery, and jugular vein may be noticed in Fig. 1. But the third and fourth aortic arches are present; the second branchial arch having within it absolutely no aortic arch, although there is a very rich plexiform communication from the bulbus aortae to the descending aorta, through the last named arch.

The ganglia of the 5th, 7th and 8th, 9th and 10th nerves are distinctly formed. The Gasserian ganglion is a large mass of epithelial cells directly under the ectoderm, external to and dorsalwards from the jugular vein. It extends over the vein and is connected, over a large area, with the brain.

The pneumogastric ganglion begins just aboralwards to the auditory vesicle, and extends downwards and backwards to the dorsal end of the second branchial furrow. The ganglion of the vagus begins still farther aboralwards, and extends to the

[1] The "cleft" is divided into two parts, external and internal. The external part is groove-like, therefore "branchial groove"; the internal part is a pocket, therefore "branchial pocket."

[2] Arch. f. Anat. u. Phys., Anat. Abt., 1887, page 9, "Nadelspitzförmige" Einsenkung.

common ectodermal depression of the third and fourth branchial grooves. The only ganglion that caused me any trouble is the ganglion of the seventh and eighth nerves. This ganglion is directly connected with the brain, extends to and is connected with the auditory vesicle, and then extends to the dorsal part of the first branchial furrow and blends with the ectoderm. This ganglion can quite easily be divided into two parts, one part belonging to the facial and the other part belonging to the auditory nerve. That the eighth nerve ganglion is connected with the auditory vesicle has already been noticed by Marshall[1] and by Froriep,[2] while the union of the facial with the ectodermal lining of the 1st cleft was first definitely made out by Van Wijhe,[3] and later by Froriep[4] and by Beard, and then by myself.[5] It is, no doubt, this union that made Balfour[6] say, "An independent origin of the facial nerve might have escaped my notice."

The lateral topography is now well made out, and now the sections must be utilized for more definite information. To describe each section is more than I can do and still spare the reader. To make a reconstruction to view the ectoderm and endoderm is about all that is left, unless a reconstruction is made for each cleft. I have found it necessary to make two reconstructions, and in each to omit the blood-vessels, for as it is, almost too many things fall in the same field.

Fig. 2 shows the direction of the two layers of the "membrane of His"[7] cut across so as to strike the branchial pockets in their deepest parts, thus forming a spiral in perspective. The grooves are represented cut across at their dorsal ends, and the ectoderm is followed around the branchial arches. Since the dorsal depressions of the branchial grooves are in a straight line (see Fig. 1), the section of the ectoderm is a plane in perspective. The whole picture is a dorsal view, therefore the fourth arch falls nearest the observer and the first arch farthest away and behind the other arches.

[1] Quarterly Journal of Microscopic Science, 1878.

[2] Arch. f. Anat. u. Phys., Anat. Abt., 1885.

[3] Van Wijhe. Ueber die Mesodermsegmente und die Entwick. d. Nerven d. Selachierkopfes. Amsterdam, 1882.

[4] Loc. cit.

[5] Studies from the Biological Laboratory, Johns Hopkins Univ., 1888.

[6] Elasmobranch Fishes. London, 1878, page 199.

[7] *Verschlussplatte.*

The line *a* (Fig. 2) outlines the roof of the mouth between the two first branchial pockets (*brp*[1]). The line then marks the deepest part of the pockets (1), then extends ventrally (*d*) between the first and second branchial arches, then across the floor of the pharynx (*c*). The second pocket is traced across the roof of the mouth in the same way, then into the second branchial pocket (2), then deep into the tissue of the ventral half of the neck (2′), and then around the third arch into the third pocket (3). The spiral is repeated to form the fourth pocket (4).

The first and second branchial pockets are much alike, both having a decided dorsal pocket and a well marked ventral pocket. The third pocket, the smallest, is somewhat triangular in shape, and unlike the first two pockets, does not quite touch the ectoderm. After the third branchial pocket is outlined to the point 3 (Fig. 2), it is naturally believed that the line should pass around the fourth branchial arch into the fourth pocket. This is not the case. In order to follow the line into the fourth pocket the line must be retraced over a mound to the point 4′, and then down a groove into the fourth pocket. The interior of the pharynx is much like the sinus praecervicalis. In the sinus the third and fourth grooves run into a common depression (Fig. 1); in the pharynx the fourth internal pocket runs into the third pocket. This being the case, the dorsal part of the fourth branchial arch can have no aortic arch, for the groove running over the branchial arch would compel the aortic arch, in case it passed through the whole length of the branchial arch, to penetrate two thicknesses of endoderm. Fig. 1 shows the relation of the aortic arch to the branchial arch. The aortic arch leaves the branchial arch before it reaches the groove that the aortic arch would have to penetrate. From this description I think that it can be made out that the upper end of the fourth pocket (Fig. 2, 4′) lies somewhat *anterior* and directly *internal* to the fourth aortic arch. The lower part of the pocket (4) must be looked upon as the pocket proper. In this embryo I can find no fossa subbranchialis as I find it in chicks[1] of this stage, and as His[2] finds it in human embryos of the same stage. Neither do I find the two-horned space as Froriep[3] has described. Froriep's

[1] Arch. f. Anat. u. Phys., Anat. Abt., 1887. [2] Ibid. 1886. [3] Ibid. 1885.

drawings show that the two horns lie lateral to the space between the fourth and fifth aortic arches.

The sections of the branchial grooves, *brf*^{I}, *brf*^{II}, *brf*^{III} (Figs. 2 and 3), show that the ectoderm is greatly thickened and turned in much as it is when the medullary canal, lens, or otic vesicle is formed. The first groove overlaps the first pocket on its dorsal side; the second groove overlaps the second pocket on its aboral side; and the common third and fourth groove (belongs more to the third than to the fourth groove) lies slightly dorsalwards and aboralwards to the third branchial pocket.

The relation of the grooves to the ganglia is given in Fig. 3. The ganglion of the vagus passes from the brain ventralwards to the "pin-point" depression of the third groove and is there directly united with the ectoderm. The ganglion of the ninth nerve takes a similar course as the tenth. The "pin-point" depression of the second branchial groove is not simply a depression but an invagination which extends directly dorsalwards and overlaps, on the anterior side, the lower part of the glossopharyngeal. In this overlapping region, the nerve and ectoderm are not absolutely united, and it was with the greatest difficulty that I separated them. The lower part of the facial nerve lies almost directly medianwards from the "pin-point" depression of the first branchial groove and is absolutely blended with it. Following this ganglionic group upwards, it is first united with the otic vesicle and then with the brain. In this embryo it was comparatively easy to separate the acusticus-facialis ganglion into its two parts, and it is the acusticus part of the ganglion that unites with the otic vesicle.

There is yet left the median thyroid gland. The gland is not fully separated from the pharynx, is already solid, and its deeper layers are rich in blood-vessels. The gland arises from a point directly upon the ventral union of the second arches, but in the lateral reconstruction (Fig. 1) the gland falls upon the first cleft because the ventral half of the second branchial arch falls more oralwards than the dorsal half of the arch does.

Embryo B.

This embryo has reached the stage of maximum flexion. The head and tail are well flexed, and the tail overlaps the interme-

diary brain. The nape-breech length is 8½ mm. and the vertex-breech length 5 mm. All the extremities have appeared, the nasal groove is well formed, and the lens is fully separated from the ectoderm. The branchial arches and clefts have grown in proportion with the rest of the body, the marked change being in the form of the second arch, which now is triangular with a very wide base. This stage corresponds well with that of a chick of four days.

The lateral reconstruction (Fig. 4) shows that, due to the extreme flexion of the head, the fore-gut has undergone a decided change. Instead of being a semicircular canal as in Fig. 1, it is now a zig-zag affair, being bent at a right angle just internal to the vagus ganglion. The bifurcation is relatively and absolutely higher than in embryo *A*. The larynx is well formed and its upper border lies directly medianwards to the fourth branchial arch. There is an embryonic epiglottis and a tuberculum impar.

The bulbus aortae retains the position it had in embryo *A*, and from it arises the third, fourth, and fifth aortic arches. The aortic arches are S-shaped, as is also the case in the chick when maximum flexion of the head has taken place.

The jugular vein now lies aboralwards to the Gasserian ganglion and makes two right angles in order to get into the thorax, where it retains the same relative position it held in embryo *A*.

As to the ganglia of the neck, it may be noted that the ganglia (seventh, ninth, and tenth) that are related to the clefts have almost outgrown the Gasserian ganglion. A glance at Fig. 1 will show how much larger the Gasserian ganglion was than the remaining ganglia, while Fig. 4 shows all four ganglia practically of the same size.

To study the clefts two reconstructions had to be made. Fig. 5 is a reconstruction of the first and second clefts, while Fig. 6 takes the region of the Sinus praecervicalis. In Fig. 6 the first pocket (*brp*[1]) has become dilated at its outer extremity. This dilatation not only extends dorsally but extends anteriorly more than again as far as it extends dorsally. From the upper, outer, and anterior part of the first branchial pocket there is a new dilatation or outgrowth connected with it, the canalis tubo-tympanicus of Kölliker,[1] and is formed much as I[2] have found

[1] Grundriss. (Sulcus t. t. of Moldenhauer.) [2] Loc. cit.

it to be formed in the chick. In this case the canalis tubo-tympanicus is bent towards the median line, therefore away from the first branchial groove. Its shape is somewhat two-horned, as is also its cavity. The ventral wall of the first pocket runs directly over into the cavity of the mouth. In the reconstruction (Fig. 5) the lateral walls of the mouth (*m*) are given.

Due to the direction of the sections, the second pocket falls higher in the reconstruction than does the first pocket. The second pocket is rectangular in shape; the uniting line (*a*) represents the dorsal line of the pharynx of a section made through the first branchial cleft. How different the second branchial cleft of this embryo is from that of a chick of the same stage of development![1] In the chick the second cleft is changed into a tube, being due to its well developed embryonic operculum.

In Fig. 5 the third aortic arch, carotid artery and descending aorta are drawn. Besides the arteries, the jugular vein is outlined as passing around the oral cavity in order to enter the head. In Fig. 6 the fourth and fifth aortic arches and descending aorta are outlined. To retain the connection between Figs. 5 and 6, the outline of the transverse section of the dorsal part of the second branchial pocket has been added to Fig. 6, anterior to the vagus ganglion. The third branchial pocket is an enormous sickle-shaped affair (Fig. 6, brp^{III}). The sickle has about the shape of the fourth aortic arch, lies mostly oralwards to it, and extends ventralwards into the branchial region to the bulbus aortae. The fourth pocket is an exaggeration of the fourth pocket in embryo *A*. To be sure, the upper groove that leads to the third pocket has mostly disappeared. It is to be noted that the fourth pocket does not extend as far laterally as it did in embryo *A*. There it extends to the outside of the fourth aortic arch; here it extends but half way over the arch. On the median side of the fourth pocket there is a depression, *f. s.* (the fossa subbranchialis), marked better in the sections than in the reconstruction.

The change required to produce the branchial region of embryo *B* from that of embryo *A* may be explained in the following way: If the dorsal part of the second cleft is fixed in

[1] Arch. f. Anat. u. Phys., Anat. Abt., Taf. II, Fig. 27.

embryo *A* and traction is made on the first cleft, with at the same time pushing force applied to the oesophagus and trachea, embryo *B* is formed. This holds as good for the external form of the embryo as well as for the branchial pockets. It may be that the aortic arches play an important part in this mechanism, for it is true that with the changes in the branchial arches the aortic arches bear a certain relation.

The "pin-point" depression of the first branchial groove is united over the canalis tubo-tympanicus with the facial ganglion. The facial ganglion is united with the brain and the auditory ganglion, which in turn is united to the labyrinth. The second groove is quite shallow throughout its whole course, the dorsal "pin-point" depression being still united with the 9th nerve ganglion (*brf*^{II}, Fig. 5).

The sinus praecervicalis (*s. pr.*) has in this embryo reached two stages of development, being more advanced on the left side than on the right. On the left side the heart extends high into the branchial region, and this in itself suffices to explain the advanced development of the left sinus praecervicalis. Besides, the head being bent to the left side possibly causes the branchial arches to appear sooner on that side, a condition that I have already noticed in the chick.[1]

On the right side, Fig. 6, the main bulk of the vagus ganglion is pear-shaped, with its stem pointing downwards. As the form of the ganglion passes over from the "body" into the "stem," it rests with the oral side of the "collar" directly upon the dorsal side of the "pin-point" depression of the third branchial groove, with which it is absolutely united (Fig. 7, *a*). The "stem" of the ganglion passes along the aboral side of the third branchial groove down to the fourth groove, being blended with the ectoderm of both grooves throughout its whole course (Fig. 6, right). A transverse section of this region, Fig. 7 (*s. pr.*), shows how the fourth groove branches off from the third, or rather how both end dorsally in the same depression.

The left side shows the same condition as the right, but in a more advanced stage. Here we can imagine that the trunk and second arch of the right side have moved toward each other and thus make the sinus praecervicalis of the left side. This now

[1] Loc. cit., p. 7.

gives us the sinus praecervicalis nearly closed off, with a deep part (fundus praecervicalis, His) composed mostly of the dorsal part of the fourth groove, and a slit-like communication (infundibulum praecervicale, His) with the exterior of the body. This infundibulum is a slit .02 mm. wide and .1 mm. long, beginning dorsally and extending somewhat anteriorly and ventrally. It is bounded dorsally by the retro-branchial ridge, aborally by the thorax, and orally by the lower border of the third branchial arch. The embryo *B* shows, that in the closure of the sinus praecervicalis, the fourth arch is first covered by the third (Fig. 7) and retro-branchial ridge, and then the third arch is mostly covered by the second. The embryo *C* shows that the fourth groove is fully closed while the third groove still communicates with the exterior of the body. This could not be the case did the second arch first cover the third and then the fourth. In the chick the whole sinus praecervicalis is closed by the embryonic operculum growing over it, but in the dog it seems as though the operculum is of still less importance than in the human embryo. I have been convinced that in the dog the fourth arch is fully covered by the third before the third is covered by the second arch. I have not been fully convinced that the second arch altogether covers the third arch, but am inclined to believe that much of the ectoderm covering the third arch aids to form the epidermis of the neck. My observation fully agrees with that of His[1] and contradicts that of Dursy[2] and Rabl.[3]

This body that is formed from the sinus praecervicalis is the body that His claims to be the thymus. A glance at my Fig. 7 and his Fig. 4 (Arch. f. Anat. u. Phys., Anat. Abt., Plate XXII, 1886) will convince any one that it is. That that body is not the thymus I think my embryo *C* will demonstrate.

Embryo C.

Nape-breech length 10 mm., vertex-breech 9 mm. The head is bent upon the body, opposite the second branchial arch, at about a right angle. The body is well curved upon itself, so that the tail nearly reaches the eye. The superior maxillary process

[1] Anatomie menschlicher Embryonen, and Archiv f. Anat. u. Phys., Anat. Abt., page 421, 1886.

[2] Entwicklung d. Kopfes d. Menschen, 1869.

[3] Prager med. Wochenschrift, 1886.

and the first and second arches are about of the same size. The first arch is thinner dorsally than ventrally. The second arch is triangular in shape, the lower border being convex, due to the embryonic operculum, while the upper border is more or less zig-zag, due to Moldenhauer's[1] colliculi branchiales externi. The third arch can hardly be made out, but the sections still give its outline. It has fully covered the fourth arch, and its ventral half is partly covered by the ventral half of the second arch.

A careful examination of the first branchial groove shows that the "pin-point" depression has not yet disappeared and that the meatus is beginning in its ventral half (Fig. 8). The second groove is marked by the operculum, being shallow dorsally and deep ventrally. The third groove is marked only by the "pin-point" depression (*S. pr.*) which runs into a very shallow groove running towards the apex of the second arch. But the fourth and fifth aortic arches remain, both being within the thoracic cavity.

The ganglia of the head have all increased in size, and they are now interwoven with nerve bundles. The Gasserian sends its three branches to the head. The seventh and eighth nerve ganglia remain much as in embryo *B*. The ganglion of the ninth nerve has doubled its length; the ganglion of the tenth more than quadrupled its length; while both have diminished their diameter by one third.

These changes are no doubt due to the stretching of the neck, the stretching being greater in the region of the sinus praecervicalis than more oralwards, for the vagus ganglion is stretched more than the glossopharyngeal, and the glossopharyngeal more than the facial. The stretching is also nicely illustrated by the position of the jugular vein. We can consider the vein of Fig. 4 fixed at two points, *i. e.* at the heart and in the head. In embryo *B* the flexion of the head upon the body is maximum, and in embryo *C* the head is in the first stage of erection. This process of erection can do but one thing, *i. e.* stretch the dorsal part of the vein in embryo *B* and produce a kinking near the heart, and a kinking as the vein passes over the seventh nerve ganglion. This is exactly the shape of the vein in embryo *C* (compare Figs. 4 and 8).

[1] Morphologisches Jahrbuch, Bd. 8.

Again, if the pharynx proper of *C* is compared with the pharynx proper of *B*, stretching is suggested. The dorso-ventral diameter of the pharnyx in *B* is wide; in *C* it is very narrow. Nothing but stretching could produce this. That a stretching of the neck takes place in the chick has already been suggested to me, for in a chick of seven days the neck is longer and thinner (?) than in a chick of six days. (See Arch. f. Anat. u. Phys., Anat. Abt., 1887, page 9, Plate I, Figs. 5 and 6.)

The head in its development flexes as long as the lower part of the body permits it to flex. During the rapid growth of the thorax the head must of necessity be lifted up, and in this lifting the ventral half of the branchial region must be stretched. During this whole time the sinus praecervicalis region is the fulcrum, and as soon as this fulcrum is broken the neck begins to elongate. But, just as the neck begins to elongate, the spinal column is becoming cartilaginous.

The sections show that the Gasserian ganglion is firmly united to the brain, the auditory united to the brain, otic vesicle and with the facial ganglion. The facial ganglion sends a large bundle of nerves into the second arch, and its old communication with the "pin-point" depression of the first branchial groove can still be seen.

In the reconstruction (Fig. 9) the first branchial pocket is outlined only in its maximum diameters. The sections show that the direction of the pocket is first directly outwards, then upwards and outwards, then bending at about a right angle and passing directly anteriorly. Now the canalis tubo-tympanicus begins, and extends, much as in embryo *B*, obliquely upwards and forwards.

The ganglion of the ninth nerve is in no way in connection with the ectoderm, the "pin-point" depression of the second branchial groove having fully disappeared.

The second branchial pocket shows more the shape it did in embryo *A*, but differs from both embryos *A* and *B* in that here it is far from reaching to the ectoderm.

As a residuum of the third cleft we have left two distinct bodies which are at no point connected with each other, and each has a distinct lumen. The anterior body is directly connected with the pharynx by a heavy band of epithelial cells which

passes between the third and fourth aortic arches. The posterior body begins within the vagus ganglion, directly posterior to the anterior body. At its beginning it has a lumen which the body retains till its ectodermal connection reaches nearly to the epidermis. On both sides of the embryo these two bodies have reached the same stage of development, and in all sections the bodies are fully separated from each other.

Fig. 10 is the left sinus praecervicalis region, taken from section No. 130. The vessels and numbers give the topography. Within the vagus nerve there is a group of cells with quite a distinct triangular outline and a lumen. Anterior to the vagus there is another group of cells which already appear in section No. 129. These two groups followed four sections deeper (.08 mm.) are again seen, Fig. 11. Here the anterior group unites with the pharynx; the posterior group is still mostly within the nerve. Two sections deeper (No. 136), Fig. 12, the anterior group has a lumen, and the posterior group communicates with the sinus praecervicalis, but its lumen does not reach to the outside of the body. A section deeper this organ of the vagus has disappeared, but its old communication with the ectoderm is marked by a band of cells which reaches nearly to the endodermal group of cells. Fig. 13, section No. 139, shows the anterior group of cells alone; it ends in section No. 144 (Fig. 14).

The reconstruction, Fig. 9, gives the relative position of the two bodies, the one from the third pocket extending more aboralwards than the one from the sinus praecervicalis. The body from the sinus is absolutely blended with the ganglion of the vagus nerve and still communicates with the ectoderm. This body from the sinus is developed from the dorsal part of the third and fourth branchial grooves. As the third arch falls over the fourth arch, a good share of the ectoderm covering the lower part of the third arch and the epithelium of the dorsal half of the fourth arch are included. This makes the triangular body that arises from the sinus praecervicalis identical with the thymus of His. It is, further, the body that Born[1] described as extending from the sinus praecervicalis into the vagus, and it is the body that Froriep[2] described as being the organ of the vagus. Besides

[1] Arch. f. Mik. Anat., Bd. 22, Seite 295.

[2] Loc. cit. See his Taf. II, Figs. III 4 to III 6, and Figs. IV 5 and IV 6.

this body, both Born and Froriep have described the thymus as arising from the third branchial pocket.

A distinct fourth branchial pocket does not exist in this embryo, but in its place there is a depression to either side of the pharynx, beginning just below the third pocket (Fig. 14, *f. s.*), and passing downwards, and anterior to the trachea. This is nicely shown in Steida's[1] Figs. 6 and 8, Fig. 8, *schd.*, showing how the aboral end of this groove passes deep into the tissue and a transverse section cuts it off, thus giving the appearance of a gland. The same is shown in Born's[2] Fig. 12.

My lateral reconstruction (Fig. 8, *f. s.*) shows the position of this diverticulum, and my dorsal reconstruction (Fig. 9) shows that the diverticulum lies internal to the fourth aortic arch. Had the sections been cut from a point higher dorsally and lower ventrally, it would have thrown this body internal to the fifth aortic arch, thus making it the fossa subbranchialis. In Fig. 9, the fossa can be seen only on the right side; on the left side it is covered by the fifth aortic arch. Froriep gives this semicircular slit in his Fig. III 6 and marks its ventral end as fourth branchial pocket. In his description (page 24) he states that in this stage (embryo 12 mm. long) the fourth pocket no longer lies between the fourth and fifth aortic arches, but medianwards from them. The communication of the fourth pocket with the pharynx is through a groove which reaches nearly to the pharyngeal opening of the third pocket. (See also his Figures III 5, III 6, and IV 6.) I do not consider it proper to call this slit the fourth pocket, for no fourth pocket remains. The pocket has fully disappeared, and now why call a slit to either side of the pharynx the fourth pocket, even if it is formed from the pocket? This groove corresponds very well with the sulcus arcuatus of His, but there is no fourth pocket for the sulcus to end in.

The median thyroid is composed of two distinct lobulated vascular lobes, bound together by a bridge which passes around the anterior part of the bulb of the aorta. There is as yet no free lateral thyroid in the tissue of the neck, but, no doubt, it is the lower part of the sulcus arcuatus which is to form it.

[1] Entwick. d. Thymus Gl. Thyroidea, etc., 1881.
[2] Loc. cit.

Embryo D.

Nape-breech length 13½ mm., vertex-breech 15 mm. The neck is now quite well formed and straight. The tissues show a firmer appearance and the future bones are all outlined. The eye is fully formed, well marked, and eyelids have appeared.

The profile view (Fig. 15) shows that the arterial system has made no marked advancement. The jugular vein lies more ventrally than formerly, the ganglionic portion of the vagus lying mostly on its dorsal side. Relatively higher in the neck is the pharynx, back of which are the well formed epiglottis and larynx.

The ninth nerve ganglion has not increased in size; the seventh and eighth nerve ganglion has increased but slightly; the Gasserian ganglion greatly; and the vagus enormously.

The thyroid is a large lobulated gland, the lobes being connected by a bridge. Just opposite the middle of the thyroid the thymus begins. It contains a lumen and extends into the thorax, where it becomes quite lobular.

With the exception of the meatus there is left no trace of the branchial grooves, and the arches can no longer be made out. The dorsal reconstruction shows a more familiar picture (Fig. 16). The asymmetry of the whole picture, blood-vessels, oesophagus, trachea and thyroid, and the twisted thymus of the right side, all attract attention.

Around the ventral half of the first branchial groove Moldenhauer's colliculi branchiales externi have well marked the form of the external ear. Between the colliculi there is a deep depression, the meatus, which is formed to such a great extent by the colliculi projecting beyond the exterior of the body that the meatus must be looked upon as being partly formed by the colliculi building a wall around a part of the branchial groove. The development of the neck has caused the meatus to be relatively nearer the spinal column than it is in embryo *C*.

From the sections it is seen that the canalis tubo-tympanicus is relatively nearer the meatus than in embryo *C*, in which the meatus is at the ventral part of the cleft and the canalis t. t. at the dorsal part. The diameter of the canalis t. t. is now narrower than before. As to the direction of the canalis, it is now not as much forward as before, for, if its direction is pro-

jected to meet the perpendicular line of the body, the lines intersect at an angle of 110°, while in embryo *C* the angle is a right angle. The whole picture is much as it is in a chick of 6 days 16 hours.

Fig. 14 (embryo *C*) shows the relative position of the thyroid, oesophagus, trachea, and fossa subbranchialis, which is yet in free communication with the pharynx. This section can easily be replaced in Fig. 8 and the topography made out. In embryo *D* a section that falls immediately below the larynx again gives nearly the same picture, with the exception that the aortic arches are not in the field, and that the fossa subbranchialis and the median thyroid fall together. This change in position is again difficult to understand unless we again imagine a stretching force to act upon the neck. All we need to do in embryo *C* to produce embryo *D* is to pull up on the larynx. This motion elongates vein, vagus, carotid, and thymus, and draws the median thyroid and fossa subbranchialis (lateral thyroid) into the same field.

The lobes of the lateral thyroid are now not a mass of indifferent cells as formerly. The thyroid can now be distinctly separated into two parts, the old median thyroid and a convoluted tubule. The picture is nicely given in Steida's Figs. 10 and 11. At no point is the thyroid connected with the endoderm.

I could not see in any of my preparations the carotid glands that Steida pictures and describes. In his Figs. 8 and 10 the carotid gland is attached to a horn of what I make out to be the fourth branchial pocket. This horn I saw at no time, but cannot deny its presence in a stage between embryos *C* and *D*. Froriep, who describes this region, carefully mentions a "three-horned" condition of the fourth pocket. The first two horns are covered with small masses of cells[1] and project laterally, while the third is a smooth slit and projects aborally. The laterally projecting pockets are no doubt the remains of the fourth pocket proper,[2] and possibly are the projections that Steida called the carotid glands. The third aboralwards projecting pocket is the fossa subbranchialis. Froriep interprets these auxiliary pockets as rudimentary branchial pockets, a view again advanced by

[1] Loc. cit., page 24, "mit Epithelwucherung besitzte Ausbuchtungen."
[2] Also Froriep's view. See his Figs. I 1 and II 5.

de Meuron.[1] That the fourth branchial pocket plays a more important part in the birds than in the mammals I have already shown.[2] In the chick the two bodies that arise from the fourth pocket and fossa seem to be homologous with the lateral thyroid gland in mammals. His states that the lateral thyroid gland arises in the human embryo from the lower part of the sulcus arcuatus, while Born, Fischelis,[3] and de Meuron place it in the fourth pocket. But I am convinced that the fourth pocket forms the lower (not lowest) part of the sulcus arcuatus, therefore, originally the lateral thyroid was located in the fourth pocket, making its origin agree very well with the origin of my corpus x.

From the first branchial cleft the Eustachian tube, tympanic cavity, tympanic membrane, and meatus are formed. The tube is formed directly from the first cleft proper, while the tympanic cavity is a new outgrowth or dilatation of the outer anterior part of the cleft. This new outgrowth that was first described by Moldenhauer[4] has been accepted by Kölliker[5] and Froriep,[6] and lately again demonstrated by Hoffmann[7] and by myself.[8] The tympanic membrane is formed directly from the membrane of His, as I have already demonstrated in the chick. Besides this, the dorsal part of the first groove dips into the tissue of the head, meets and is absolutely blended with the ganglion of the seventh nerve. Whether this involution forms the whole ganglion, or whether it only unites with the already existing ganglion, I cannot answer, for in my youngest embryo the ganglion and ectoderm are already blended. This union is still present in embryos *B* and *C*.

The blending of the ectoderm of the first cleft with the facial

[1] Recherches sur le développement du Thymus, etc. Inaug. Diss. Genève, 1886.

[2] Arch. f. Anat. u. Phys., Anat. Abt., 1887.

[3] Arch. f. Mik. Anat., Bd. 25.

[4] Moldenhauer. Morphologisches Jahrbuch, 1877.

[5] Kölliker. Entwicklungsgeschichte d. Menschen, etc., 1879. Grundriss, 1884.

[6] Froriep. Loc. cit.

[7] Hoffmann. Arch. f. Mik. Anat., 1884.

[8] Mall. Studies from the Biological Laboratory, Johns Hopkins University, Vol. IV, 1888.

nerve was first described by Van Wijhe[1] in the selachian embryo, and has since then been verified in the mammalian embryo and the chick.[2]

The ganglion of the eighth nerve is in all the stages connected with the epithelium of the auditory vesicle, as demonstrated by Marshall[3] and Froriep.

The glossopharyngeal nerve ganglion blends with the dorsal part of the second branchial groove. The blending is beginning in embryo *A*, is complete in embryo *B*, and is again separated in embryo *C*.

The region of the sinus praecervicalis is of most interest, for it is from the sinus that His states that the thymus arise. Already in embryo *A* the common depression of the third and fourth branchial grooves is blended with the vagus ganglion. In embryo *B* two stages of development of the sinus praecervicalis are present. On one side the sinus is quite deep, while on the other side the sinus is nearly closed off, forming the infundibulum praec. and fundus praec. of His. In the closing off of the deeper part of the sinus the dorsal part of the fourth arch is included, as described by His. In embryo *C* the ectoderm of the sinus is nearly closed off from the exterior of the body, the connection being a solid band of epithelial cells. In embryos *B* and *C* the fundus praecervicalis is absolutely blended with the lower part of the vagus ganglion. In embryos *A* and *B* there was absolutely no formation from the third branchial pocket. The third pocket was yet a slit, and at no point was there a thickening of the epithelium. But in embryo *C*, where the vagus organ (sinus) is just about separated from the ectoderm, the third pocket is converted into a triangular body which lies close upon and is anterior to the vagus. These two bodies (see Figs. 9, and 10 to 14) are fully separated from each other, and each has a lumen. The body from the sinus praecervicalis extends into the vagus nerve and is absolutely blended with it. The body from the third pocket lies anterior to the vagus organ, communicates with the pharynx and extends relatively

[1] Van Wijhe. Loc. cit.

[2] Beard, Zool. Anzeiger, 1884. Froriep, Arch. f. Anat. u. Phys., Anat. Abt., 1885. Kastschenko, Arch. f. Mik. Anat., Bd. 30. Mall. Kastschenko, Arch. f. Anat. u. Phys., Anat. Abt., 1887. Beard, Studies from the Biol. Lab., Owens College, Vol. I.

[3] Marshall. Quarterly Journal of Microscopical Science, 1878.

deep into the thorax. In embryo *D* the vagus organ has fully disappeared, and the organ from the third pocket remains in the position that it occupied in embryo *C*.

What the ectodermal involutions of the branchial grooves to the 7th, 9th and 10th nerves mean I shall not attempt to say. Froriep says, page 41: "The position of these rudimentary sense-organs is the same for the three nerves; the dorsal part of the branchial clefts. They may, therefore, for short, not improperly be called the 'organs of the branchial clefts.'" Page 45 he continues: "The branchial cleft-organs form no definite structure for the facial, glossopharyngeal, or vagus nerves; what is left of them is solely the ganglionic swelling of the nerves, which originally was the ganglionic base of the sense epithelium. These ganglia, ggl. geniculi, ggl. petrosum, and ggl. nodosum, are therefore to be viewed as rudimentary organs." This view is also brought out by Beard and is accepted by Kastschenko. There is, however, no evidence in calling them "sense-organs."

Can it now be possible that the organ from the third pocket comes and disappears and that the organ of the vagus leaves the vagus and forms the thymus? Prof. His's error no doubt lies in the fact that the sinus praecervicalis is long in developing. It forms a very conspicuous body in the region of the thymus. Just as this body from the sinus is disappearing, the thymus rapidly appears and takes its place. The thymus once formed, grows rapidly, and, therefore, apparently, the former rapidly developing and conspicuous sinus praecervicalis is converted into the thymus.

I have again studied my chick collection and again make my former statement, *i. e.* that the thymus arises from the third branchial pocket. The thymus is already beginning in a chick of 3 days 7 hours as a slightly dorsal outgrowth from the third branchial pocket. This outgrowth gradually increases in embryos of 3–16, 3–22 and 4–3 hours, till in the embryo of 4–16 the thymus is being separated from the pharynx. (See Arch. f. Anat. u. Phys. 1887, Plate II, Fig. 27.) The sinus praecervicalis does not play the important part it does in the mammalian embryo. In a chick of 4–16 hours I could make out quite a definite union between vagus and third branchial groove. In the chick of 4–3 hours this union is severed, but is still marked by a row of spindle cells passing from the vagus to the third branchial pocket.

Dohrn,[1] and later Maurer,[2] demonstrated that the thymus of fishes arises from the dorsal end of the branchial clefts. This seems to prove that the thymus arises from both ectoderm and endoderm, but Balfour[3] has shown that the cleft proper is lined altogether with ectoderm.

The different views regarding the origin of the thymus in the mammalian embryo are already known. It was for a long time disputed whether or not the thymus arises from epithelium. Arnold,[4] who was the first to locate the origin of the thymus, maintained that it arose in common with the thyroid from the epithelium of the pharynx. This statement was denied by Bischoff,[5] then first confirmed and then denied by Remak.[6] Remak's last view of the origin of the thymus settled to most minds that the thymus arises from the mesoderm. In two elaborate papers that appeared lately, one by Afanassiew[7] and the other by Watney,[8] this view is confirmed.

Kölliker's[9] investigations on rabbit embryos led him to believe that the thymus arises from one of the branchial clefts, because a transverse section of a young thymus looked exactly like a transverse section of a branchial cleft. Steida[10] studied many embryos and was the first to prove that the thymus arises from a branchial cleft. Born[11] studied all the branchial clefts carefully, and according to his ingenious method made models of this region. In this way he was able to definitely locate the origin of the thymus in the third branchial pocket. His work has been confirmed by Kölliker,[12] Froriep,[13] and de Meuron.[14]

[1] Dohrn. Mitt. aus d. Zool. Station zu Neapel, Bd. 5.
[2] Maurer. Morpholog. Jahrbuch, Bd. 11 u. 13.
[3] Balfour. Comparative Embryology, 1881, and Elasmobranch Fishes, 1878.
[4] Medicinisch-chirurgische Zeitung, 1831.
[5] Entwicklungsgeschichte, 1842.
[6] Entwicklung d. Wirbelthiere, 1855.
[7] Arch. f. Mik. Anat., 1877.
[8] Phil. Trans., 1882.
[9] Entwicklungsgeschichte, 1879, page 875.
[10] Untersuchungen über d. Ent. d. Gl. Thymus, etc., 1881.
[11] Arch. f. Mik. Anat., 1883.
[12] Grundriss, 1884, page 369.
[13] Arch. f. Anat. u. Phys., Anat. Abt., 1885.
[14] Recherches sur le développement du Thymus, etc. Inaug. Diss. Genève, 1886.

Professor His's[1] careful study of the sinus praecervicalis leads him to the conclusion that it forms the thymus gland. Fischelis,[2] and lately Kastschenko,[3] have come to the conclusion that the thymus arises from both the ectoderm and endoderm. In a remarkable way Kastschenko explains the origin of the thymus. According to him, it arises in the mammalian embryo from the sinus praecervicalis, as well as from the third branchial pocket. The fourth branchial pocket produces the lateral thyroid gland. In the chick, on the other hand, the thymus arises from the third cleft, the fourth branchial furrow, the fourth branchial pocket, and the rudimentary branchial pocket (fossa subbranchialis). Kastschenko's main argument for the chick's thymus is based upon an embryo of about five days (see his Fig. 20), in which he allows both ectoderm and endoderm to aid in the formation of the triangular gland that arises from the third branchial cleft. And then in an embryo of seven days (K.'s Fig. 24) Kastschenko notices that the thymus is double in form and extends around and back of the carotid artery and jugular vein. It must be taken into consideration that the later growth has nothing to do with deciding the origin of a gland. Long before the third branchial pocket is converted into a mass of cells—thymus—it sends a hollow prolongation from its outer extremity upwards and backwards. This pointed process lies lateral to the third and fourth aortic arches, and at no time is it blended with the ectoderm (see my plate II, Arch. f. Anat. u. Phys., Anat. Abt., 1887). This sack arising from the third pocket is so conspicuous in chicks of from three to six days that it cannot be mistaken. It was already described twenty years ago by His,[4] and was later again taken up by Seessel.[5] It is this body, and only this body, that forms the gland in the chick, first described by Ecker[6] as the thymus, and since then generally accepted as the thymus.

The median thyroid arises from the endoderm covering the median union of the two second branchial arches. Before it is

[1] Anat. Mensch. Embryonen, Theil III, 1885. Arch. f. Anat. u. Phys., Anat. Abt., 1886.

[2] Arch. f. Mik. Anat., Bd. 25.

[3] Arch. f. Mik. Anat., Bd. 30. Arch. f. Anat. u. Phys., Anat. Abt., 1887.

[4] His. Untersuchungen über die erste Anlage des Wirbelthieres. Leipzig, 1868.

[5] Arch. f. Anat. u. Phys., Anat. Abt., 1877.

[6] Icon. phys.

separated it is solid (embryo *A*), then separates from the endoderm and becomes lobulated (embryo *B*), and finally is divided into two distinct lobes which are united by a bridge around the anterior side of the larynx (embryo *C*). The lobes now lie directly under the lower part of the sulcus arcuatus (fourth pocket pulled out from between the fourth and fifth aortic arches, and the fossa subbranchialis). In embryo *D* the lobes of the thyroid contain distinct gland-like bodies which undoubtedly arise from the lower part of the sulcus arcuatus. In the chick the fourth pocket and the fossa subbranchialis each forms a distinct body, while in the mammalian the fourth pocket is pulled out from between the aortic arches and thus forms the sulcus arcuatus. From the lower part of the sulcus the lateral thyroid arises.

The most typical of the head segments are within the branchial region. Each segment is composed of a nerve, a muscle plate, and an artery. The segments are separated ventrally by the branchial clefts. The nerve as it is separated from the neural crest unites with the ectoderm of the dorsal part of the branchial cleft. This union remains for a considerable time and forms the "branchial sense organs" of the authors. But, besides this union, the cleft forms glands which, if Balfour's research in elasmobranch fishes is correct, must arise from the endoderm. In those animals in which the clefts do not break through, these glands arise from the branchial pockets. Viewed from this standpoint it makes it highly improbable that the ectoderm that unites with the ciliary ganglion, with the Gasserian ganglion, with the ganglion of the seventh nerve, with the ganglion of the ninth nerve, and with the ganglion of the tenth nerve, should, in the region of the sinus praecervicalis, leave the vagus and form the thymus, or even aid in forming the thymus.[1]

Febuary, 1888.

[1]After the above paper was completed and in the hands of the publisher, I received the preliminary report of an investigation by G. A. Piersol (Ueber die Entwicklung der embryonalen Schlundspalten und ihre Derivate bei Säugethieren. Sitzungsberichte der Würzburger Phys.-medic. Gesell., April, 1888). Piersol finds that, in rabbit embryos, the thymus arises mainly from the third branchial pocket, and from his own investigation, as well as from a phylogenetic standpoint, excludes the ectoderm. This statement confirms mine made in my preliminary report (The Branchial Region of the Dog, Johns Hopkins University Circular, February, 1888).

EXPLANATION OF THE FIGURES.

PLATES XIX, XX, AND XXI.

In the figures the following abbreviations are used:

a^1, a^2, a^3, a^4 and a^5. Aortic arches.
a. d. Descending aorta.
a. sub. Subclavian artery.
a. v. Auditory vesicle.
B. A. Bulbus aortae.
brf^I or brg^I, brg^{II}, brg^{III}, brg^{IV}. Branchial grooves.
br. Bronchus.
brp^I, brp^{II}, brp^{III}, brp^{IV}. Branchial pockets.
c. i. Internal carotid.
c. t. t. Canalis tubo-tympanicus.
ec. Ectoderm.
en. Endoderm.
f. s. Fossa subbranchialis.
fun. prae. Fundus praecervicalis.
G. Glottis.
g. n. Ganglion nodosum.
in. prae. Infundibulum praecervicale.
L. Lens or larynx.
M. Mouth.
M. ex. Meatus aud. externus.
n^5, n^7, n^8, n^9, and n^{10}. Cranial nerves.
o. p. Olfactory pit.
Ph. Pharynx.
S. Thyroid gland.
Sup. max. Superior maxillary process.
S. pr. Sinus praecervicalis.
T. Thymus.
Tk. Trunk.
Tr. Trachea.
v. j. Jugular vein.
v. sub. Subclavian vein.
I, II, III, IV, V. Branchial arches.

FIG. 1.—Profile reconstruction of embryo *A* (nape-breech length 6 mm.). × 25 diameters.

FIG. 2.—Dorsal reconstruction of the branchial region of embryo *A*. × 50 times.

FIG. 3.—The same, only that the endoderm is omitted and the nerve ganglia added.

FIG. 4.—Profile reconstruction of embryo *B* (8½ mm. long). × 25 times.

FIG. 5.—Dorsal reconstruction of the first and second branchial clefts of embryo *B*. × 50 times.

FIG. 6.—The same for the third and fourth br. clefts. × 50 times.

FIG. 7.—Section through the branchial region of embryo *B*. × 25 times.

FIG. 7 *b*.—A reconstructed sagittal section through the point where the ectoderm blends with the vagus nerve ganglion. Embryo *B*. × 25 times.

FIG. 8.—Profile reconstruction of embryo *C* (10 mm. long). × 25 diameters.

FIG. 9.—The same, dorsal view of branchial region. × 25 times.

FIGS. 10 to 15.—Sections through the thymus and fundus praecervicale of embryo *C*. × 40 times. The sections are .02 mm. thick.

FIG. 10 is section No. 130.
11 " " 134.
12 " " 136.
13 " " 139.
14 " " 144.

FIGS. 15 and 16.—Profile and dorsal reconstructions of embryo *D* (13½ mm. long). × 25 times.

PLATE XX.

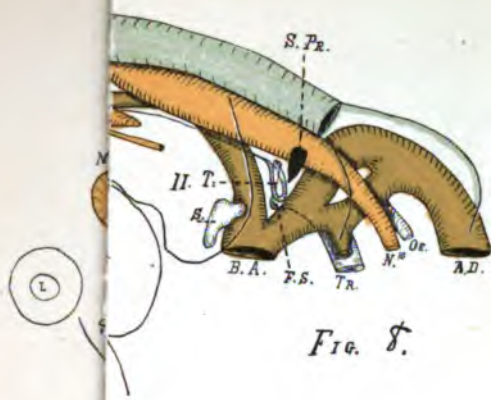
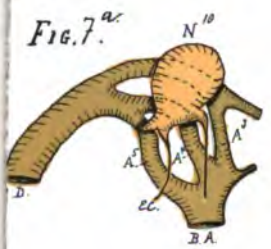


FIG. 8.

FIG. 7.a.



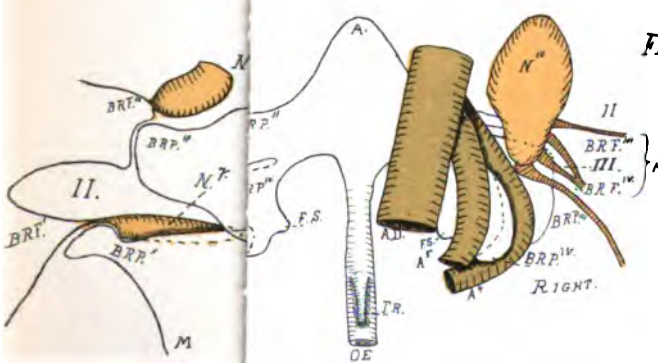
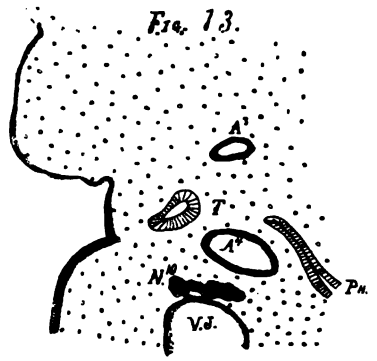


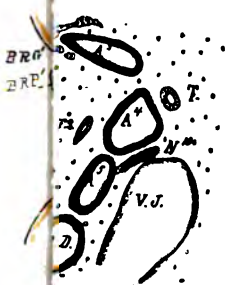
FIG. 6.

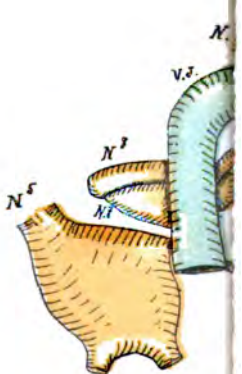
PHOTO. LITH. BY A. HOEN & CO. BALTIMORE, MD.

Fig. 10.

Fig. 13.







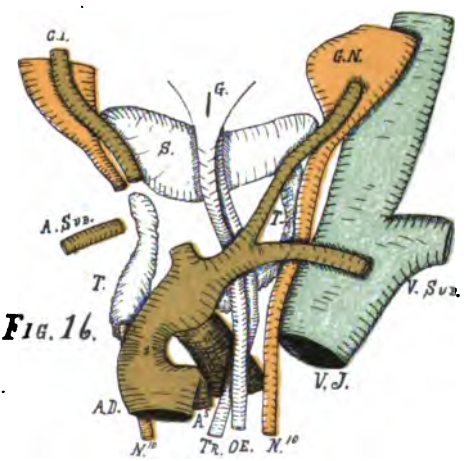


Fig. 16.

S. WATOSE Del.

PHOTO. LITH. BY A. HOEN & CO. BALTIMORE, MD

EXPERIMENTS WITH CHITIN SOLVENTS. By T. H. MORGAN.

In the *Zoologische Anzeiger* for 1885 (Vol. VIII, p. 333) there appeared a short article entitled "Neue Lösungsmittel des Chitins." These solvents are Labaraque solution (potassium hyperchlorite), and Javelle solution, the corresponding sodium compound.

It is there given that the hardest chitin parts of insects, or of other animals, may be dissolved completely by either of these solutions, preference being given to the potassium compound. One also finds that a solution of either of the above, diluted four to six times with water, will bring about changes in the chitin not visible perhaps on the exterior, but by means of which the brittleness of the chitin is removed and the easy penetration of staining fluids brought about. In conclusion, the author, Dr. Loop, says these compounds need to be further investigated.

During the winter of 1887–8 I have made use of these fluids to quite a considerable extent, and the following results may, I hope, be found of use in dealing with parts of insects or other animals which contain chitin.

General Application.—It so happened that the first experiments were made upon the eggs of the common cockroach, and the selection turned out in the end to be a most fortunate one. A great many eggs are laid at one time, the whole number being surrounded by a stiff chitinous coat, forming the so-called raft. A few words are here needed as to the strength of the solution to be used. There are two principal methods of application. The commercial fluid will be called the strong solution in the following account; and the same fluid, diluted from five to six times with water, will be called the weak solution. Later the more special application of the liquids will be discussed.

From a very great number of experiments the following examples which have proved the most successful on the roach's eggs are taken.

1. The rafts were placed, while in the fresh condition, in a weak solution of the Labaraque compound, and left until the

chitinous envelope became soft and transparent. The time varies; if *slightly warmed* the time is less, for the warm solution perhaps thirty minutes to one hour; but one must go more by the appearance of the chitin than by any definite time. If the embryos are far advanced they may now be removed from the envelope one by one; if still young, they had better be hardened and cut all together. In both cases the eggs or embryos were next washed for a few minutes in water, and then transferred for an hour to picro-sulphuric acid, then as usual they are passed through the grades of alcohol, 70 per cent, 80 per cent, 95 per cent. Here they may be kept indefinitely. When wanted for cutting, they are carried through absolute alcohol, then turpentine or chloroform, and embedded in paraffin in the usual manner. They were next cut on the microtome, fixed to the slide by either the collodion, albumen, or alcohol method, and stained, as it is called, "on the slide."

Several variations of this method suggest themselves. The strong solution was at times used, but its action is of course much more rapid and powerful. Instead of picro-sulphuric acid one may use a solution of corrosive sublimate, and this proved almost as successful as the former hardening reagent. Or again, chromic acid was used with fair results. Or the softened substance may be taken from the water and run immediately through the different strengths of alcohol, and here again one gets about as good preparations as from the picro-sulphuric acid.

2. To specimens which have been already hardened and preserved the solvent may also be applied; but in all cases where fresh material is easily obtainable, it should immediately have its chitin softened and then afterwards be preserved. Here the method is somewhat shorter, since the substance has been previously hardened. From alcohol—weak solution—they are put into the Labaraque and softened as above, then passed through water and the alcohols, etc.

In most cases in which an animal egg or embryo is encased in chitin, the best results have been obtained by staining the sections after they have been cut and fixed to the slide. If the specimen is small, staining *in toto*—after having the chitin softened, or if before this has taken place, after having made an entrance through the chitin with a point of a needle—is equally good. The greatest difficulty, and practically the only one which

one meets with, is that the Labaraque solution not only attacks the chitin itself, but after a time the soft tissues of the animal—apparently the connective tissue. Where the chitin surrounds the object completely, as in the case with the roach's raft, one can remove the object from the solution as soon as the chitin is softened and before the underlying parts have been attacked. In cases like this the solvent is at its best.

Very often, however, the soft tissues of the animal are exposed in places between the chitin covering. This is well illustrated by the joints of insects' legs, etc., and very frequently these exposed places are attacked before the chitin is completely softened, thus causing the joints, if much handled, to fall apart.

By judiciously diluting the solution and taking the parts to be softened from it before the joints are attacked, one will find its application practicable even here.

The greatest difficulty of all is when the chitin is internal, completely surrounded by soft tissue. So far as I have made any experiments here, I find that one gets better results with very dilute solutions—diluted from eight to ten times, or even more. It must be admitted that in this last case the application of the solvent is more doubtful, and of not nearly so much service as in the first and second supposed cases.

Strong solutions, then, had better be used only when the chitin completely surrounds the soft animal parts, and dilute solutions must be used in all cases where these latter substances are exposed. The solution not only softens the chitin, but removes all pigment either in the chitin or in the tissue beneath, and this is at times advantageous. Dr. Loop suggests in his article that chlorine is the active agent in bringing about these changes. Acting upon this suggestion, a few experiments were made with a strong solution of chlorine water; but although the chitin was to some extent affected, this took place very slowly and incompletely, showing that something else in the compound besides free chlorine is brought into play.

The greatest difficulties have always been experienced by those who, in making sections, have had to deal with chitin; and while these solutions cannot in every case be used to advantage, yet I believe that in a very great number of cases they will be highly useful, and by their application difficulties which have been heretofore practically insuperable may be overcome.

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SOME OBSERVATIONS ON THE EFFECT OF LIGHT ON THE PRODUCTION OF CARBON-DIOXIDE GAS BY FROGS.

By H. NEWELL MARTIN, M. D., F. R. S., and JULIUS FRIEDENWALD, B. A.

The influence exerted by light on the metabolisms of the animal body has been recognized for some fifty years. The first thorough and carefully executed work on this subject was published by Moleschott.[1] That writer concluded from a large number of experiments on frogs, that these animals expire, under similar temperature conditions, $\frac{1}{13}$ to $\frac{1}{4}$ more carbondioxide in the light than in the dark; that the greater the intensity of the light, the greater the quantity of carbondioxide produced; and furthermore, that this action of the light is effected not only through the eye but also through the skin. Since the publication of Moleschott's work, many observers have investigated this phenomenon. In general the conclusion has been reached that animals of the same species, kept at uniform temperature, expire for each gram of body-weight more CO_2 per hour in light than in darkness, and that the quantity of carbondioxide produced varies with the temperature, the intensity and color of the light, and the species of animal employed.

As it had been demonstrated that the increase in the production of carbondioxide brought about by light is effected mainly through the optic nerve, we endeavored to determine during the months of February, March, April and May whether this action is merely reflex, or whether it depends on psychical conditions in which the cerebral hemispheres are concerned. For this purpose the cerebral hemispheres of a dozen frogs of the species Rana Catabiana (nearly all of the frogs weighed over 100 grams) were removed, care being taken to avoid touching or in any way injuring the optic nerves. The frogs were then put aside and carefully watched until the wounds had healed. None of the frogs were used in any of our experiments until a month after the operation had been performed, at a time when all traumatic inflammation had passed off. In every case a post-mortem examination was made to determine whether the optic nerves had been cut or injured.

[1] Wiener Med. Wochenschrift, 1855.

The apparatus used for measuring the quantity of carbondioxide produced was in the main similar to that employed by Moleschott. The frogs were placed within a bell-jar, the bottom of which was immersed in a shallow vessel containing mercury. The air led to the bell-jar passed through a flask containing a strong solution of caustic potash, connected with which was a U-tube filled with caustic potash, which led to a similar U-tube communicating with the bell-jar. Connected with the exit side of the bell-jar was a flask containing concentrated sulphuric acid, followed in turn by a U-tube containing bits of calcium chloride, a weighed caustic potash U-tube, a weighed Geissler's bulb with its caustic potash, two more weighed caustic potash U-tubes, another calcium chloride U-tube, a flask containing a solution of barium hydrate, another calcium chloride tube, and finally an aspirator. The air, freed of all carbondioxide, entered the jar containing the frogs. From here the carbondioxide given off by the frogs passed through the sulphuric acid and calcium chloride, where it was freed of all moisture, and thence entered the weighed caustic potash tubes, by which it was absorbed. The baryta bulb placed beyond these tubes to indicate whether all the carbondioxide had been absorbed by the caustic potash tubes never showed the slightest trace of a precipitate. All the connections were hermetically sealed, so that no air could enter nor gas escape except through the regular channel, and tests were made in this respect until we were confident that this end had been attained. The apparatus was placed in the "dark room" of the biological laboratory, which could be made perfectly dark or moderately illuminated at pleasure. The caustic potash bulbs having been carefully weighed, the frog was weighed and placed under the bell-jar, the aspirator was started and the current of air regulated, and thus the experiment began. Each experiment lasted twelve hours, and hourly the temperature of the room was taken and an average made. After the experiment the caustic potash bulbs were again weighed, and from the weight of the animal and the weight of the carbondioxide given off, the quantity of carbondioxide produced was calculated for twenty-four hours for each hundred grams of frog.

The first experiments were performed with normal frogs, and Moleschott's conclusion was confirmed that these animals expire more carbondioxide in the light than the dark. The actual figures

are given in the subjoined table. Twenty-six experiments (13 of which were in the light and 13 corresponding ones in the dark) were performed on frogs deprived of their cerebral hemispheres. Of these it will be observed that in only two instances (experiments Nos. 4 and 7) was there an excess of carbondioxide obtained in the dark; and as the frogs used in these instances were very small, weighing respectively 44 and 42 grams, it can readily be understood how an error might arise in dealing with the very small quantities of carbondioxide given off by these frogs. In all other instances the quantity of carbondioxide was found to be greater in the light than in the dark. All experiments bracketed together were performed on the same frog, and it is worthy of mention that much less carbondioxide was given off on rainy or cloudy days in the light than on bright days. Thus in experiments 14, 15 and 16 we find the figures 848 and 852 on bright days, while 678 was obtained on a cloudy day.

It is also interesting to note, that comparing the quantities of CO_2 obtained from frogs with their hemispheres removed with those obtained from normal frogs, we find that most of the former figures fall within the maximum and minimum limits of the latter. Making the same comparison of the former figures with those obtained from normal frogs by Moleschott, all of the latter also fall within those limits; from which we may conclude that the cerebral hemispheres exercise no control on the oxidations in the frog's body.[1] This is well shown in experiments 2, 3 and 11, in which the same frog was used. This frog was normal in experiments Nos. 2 and 3, and afterwards its cerebral hemispheres were removed.

Our next endeavor was to determine whether, in frogs deprived of their cerebral hemispheres, the light acts only through the eye or also partly through the skin. For this reason a frog was blinded by removing its eye-balls, and the quantity of carbondioxide given off in the light and dark determined. From a glance at the table it is evident that under these conditions a greater quantity of carbondioxide was obtained in the light than in the dark, although our differences in this regard are not as great as those obtained by Moleschott. This less difference may

[1] Corin and Beneden came to a similar conclusion, working on pigeons. Archives de Biologie, Tome VII, Fasc. II, 1887.

be due to the fact that our frogs when exposed to light were in a room with walls painted black, and therefore not very brightly illuminated.

As the result of our experiments we believe we are justified in concluding that in frogs deprived of their cerebral hemispheres a greater quantity of carbondioxide is given off in the light than in the dark; that therefore the influence of light in producing greater oxidations in normal frogs is simply reflex, and not due to greater bodily activity brought about by psychical conditions dependent on the light; that the cerebral hemispheres do not take any direct part in regulating the oxidations of the frog's body; and that this reflex action of the light, though mainly effected through the eye, is produced partly also through the skin.

TABLE.

No. of Experiment.	Date. Light.	Date. Dark.	Temperature °C. Light.	Temperature °C. Dark.	Quantity of CO_2 in mgr. given off in 24 hours for 100 grams of frog. Light.	Quantity of CO_2 in mgr. given off in 24 hours for 100 grams of frog. Dark.	
1	Feb. 23	Feb. 24	18.4	18.9	764	722	Normal frogs.
2	" 28	" 29	19.2	20.	678	440	
3	March 1	March 2	19.3	20.	652	428	
4	" 5	" 6	19.5	20.2	426	442	Frogs deprived of their cerebral hemispheres.
5	" 8	" 9	20.6	19.2	766	658	
6	" 12	" 13	18.5	21.5	798	648	
7	" 15	" 16	20.	20.5	400	422	
8	" 19	" 20	18.8	17.5	724	524	
9	" 22	" 23	17.9	17.4	680	534	
10	" 26	" 29	18.6	21.5	592	423	
11	April 3	April 5	19.5	19.	590	438	
12	" 10	" 12	17.6	18.9	344	338	
13	" 17	" 19	17.83	18.	682	436	
14	" 24	" 26	18.45	24.63	848	708	
15	May 1	May 3	17.16	20.02	852	696	
16	" 8	" 10	24.41	24.50	678	656	
			Average,		644	533	
			or as		1.20	: 1	
17	May 20	May 19	18.	18.5	820	792	Frogs deprived of their cerebral hemispheres and also blinded.
18	" 21	" 20	18.5	19.3	846	798	
19	" 22	" 21	19.5	19.6	826	790	
			Average,		830	760	
			or as		1.09	: 1	

ON THE COMPARATIVE PHYSIOLOGICAL EFFECTS OF CERTAIN MEMBERS OF THE ETHYLIC ALCOHOL SERIES (CH_4O to $C_5H_{12}O$) ON THE ISOLATED MAMMALIAN HEART.

By JOHN C. HEMMETER, M. D.

The literature on the subject of the physiological activity of alcohols is very extensive; its abnormal growth appears to those who study and make an effort to keep abreast of the progress and advancement of experimental therapeutics, out of all proportion to any real increase in our knowledge of the subject. In attempting, therefore, a very brief review of the scientific work that has been done in this direction, all speculative literature is excluded.

One of the earliest series of investigations on the comparative physiological effects of alcohols is reported in Liebig's Annalen (d. Chemie u. Pharmacie, LXXIII, p. 213) for 1850, by T. Schlossberger, who worked under Griesinger. This report is very incomplete, stating simply that chemically pure methyl and amyl alcohol were experimented with, and that their action is similar to that of ethyl alcohol. As regards intensity of action, amyl alcohol did not exceed ethyl and methyl alcohol. The animals experimented upon were cats, dogs, and rabbits. The statement of method employed and of the results is quite unsatisfactory.

Nothnagel and Rossbach, in their "Handbuch der Arzneimittellehre" (1880, p. 340), give an account of experiments with the first five members of the ethylic alcohol series. The action of all is said to be similar in quality but differing in quantity. The poisonous property increases with the molecular structure, being least marked with methylic and most marked with amylic alcohol. On the authority of a thesis by A. F. A. Gros, "L'action de l'Alcool amylique sur l'Organisme," Strassbourg, 1863, Nothnagel and Rossbach give a definite numerical relationship, stating amylic to be thirty times as poisonous as methylic and fifteen times as poisonous as ethylic alcohol. From this one would

be justified in concluding that Gros found ethyl to be fifteen times stronger than methyl alcohol.

Dujardin Beaumetz and Audigé have published a series of investigations on the comparative toxic properties of the alcohols, in Compt. rend. LXXXI, p. 152–154, and LXXXIII, p. 80, which are abstracted as follows in the Journal of the Chemical Society, Vol. 29, 1886, and Vol. 30, p. 539. "These experiments consisted in administering varying proportions of ethylic, propylic, butylic, and amylic alcohols to various dogs, the quantities in some cases being passed into the stomach, in others injected under the skin. The weight of the doses administered was in every case kept in ratio to the weight of the animal operated upon. The following conclusions are drawn from the results:

"1. The toxic properties of fermentation alcohols follow their atomic composition in a sort of mathematical order. The higher the figures representing the atomic composition, the more considerable is the toxic effect. This is equally true whether they are injected under the skin or introduced into the stomach.

"2. For the same alcohol the toxic action is greater when the dose is administered through the stomach than subcutaneously; in the latter case the toxic effect is increased by dilution.

"3. The toxic phenomena are the same for all alcohols, save in the degree of intensity. The injuries caused follow in a progressive scale from the ethylic to the amylic alcohol. The injuries to the mucous membrane of the stomach being just as great whether the alcohols are administered subcutaneously or are ingested by the stomach. Severe congestion of the small intestines was noticed in some cases, in whichever way the alcohols had been administered; and, moreover, it was noticed that for the same alcohol, the congestion and pulmonary apoplexy were most frequent when the alcohol had been administered by the stomach."

In the "Bulletin gen. de thérap.," September 30, 1884, p. 251, Dujardin Beaumetz and Audigé gave the following table of doses of alcohols per kilogram weight of animal, causing death in 24 hours:

	Pure.	Diluted.
Ethyl,	8.00 grams.	7.75 grams.
Propyl,	2.90 "	3.75 "
Butyl,	2.00 "	1.85 "
Amyl,	1.70 "	1.50–1.60 "
Methyl,	... "	7.00 "

Dujardin Beaumetz and Audigé's experiments have been in part repeated by Stenberg (Sten Några experimentela bidrag till belysande af frågan om det inflytaupe, som bräuvinets föroreningar hafrapå dess fysiologiska verkningar), Nordiskt Med. Arkiv, B. X, N. 21.

This observer believes that the methods of the French investigators have led them to defective results. By injecting the alcohols subcutaneously in the doses used by them, he found them to cause great pain, irritation and suppuration. He holds that the amount of glycerine with which Dujardin Beaumetz and Audigé mixed the alcohols before subcutaneous injection is itself sufficient to cause death.

In Pflüger's Archiv for 1874, Vol. VIII, p. 605, are published a series of résumés of experiments by J. Dogiel, which are often referred to as proving that alcohol really stimulates the heart and raises blood-pressure. According to Wood's Handbook of Therapeutics, it is affirmed by Dogiel that arterial pressure is at first increased and then diminished. During the latter state the vaso-motor centres are found to be incapable of responding to stimulation. The rate of the heart-beat is stated to be at first increased, then diminished, then increased; the first increase being owing to the stimulation of the accelerators, the diminution to stimulation of the vagus, and the final increase to paralysis of the same. This experimental evidence seems to Wood to confirm the clinically known fact that alcohol in moderate amounts powerfully stimulates the heart and circulation, but in poisonous doses diminishes both the force and frequency of the pulse.

Another contribution to our knowledge of the physiological action of alcohol which has scientific merit is that of Dr. H. Zimmerberg (Untersuchungen über den Einfluss des Alcohols auf die Thätigkeit des Herzens. Dorpat, 1869). These studies were carried on with men and frogs, rabbits and cats. "Zimmerberg asserts that alcohol causes a decided reduction of the pulse rate and of arterial pressure. If the vagi were cut during this depression, a sudden rise beyond the normal point in both pulse rate and arterial pressure occurred. When the alcohol was injected towards the heart into the jugular vein, the fall in the arterial pressure was almost instantaneous and very marked.

After section of the vagi the alcohol lessened the arterial pressure without affecting the pulse rate." As these observations are opposed to clinical experience, it was presumed that some fallacy must be underlying them, which was, by Wood, found in the enormous doses of alcohol used by Zimmerberg. According to Dr. J. W. Warren (Alcohol Again, Boston Medical and Surgical Journal, July 7 and 14, 1887), the dissertation of Zimmerberg does show large doses for frogs, rabbits, and cats, but it cannot be urged that those given to men were unduly large. On the whole, it seems to us that in some of his conclusions Zimmerberg has the weight of later evidence in his favor.

It will not prove expedient to quote here the experiments of Parkes and Wollowicz upon man *in extenso;* they show the accelerating effect upon the heart-beart by sphygmographic tracings, which, however, give no distinct indication of increased arterial pressure. These results are similar to those of Dr. Edward Smith (On the action of foods on the respiration during the primary processes of digestion. Phil. Trans., Vol. 149, p. 731). The experiments of Parkes and Wollowicz were not carried out with pure alcohol, but with brandy, and the results of both of these observers, together with those of Dr. Edward Smith, have been partially corrected by experiments made with pure ethyl alcohol, by Martin and Stevens, and published in a previous number of this Journal, and the present series of experiments are a continuation, or rather an outgrowth from those made by the last named workers.

We come now to speak of a series of experiments by Sydney Ringer and Harrington Sainsbury, published in Vol. 30, 1883, p. 339 of the London Practitioner (Observations on the relative effects of certain members of the ethylic alcohol series on the ventricle of the frog's heart). "The object of these experiments was to determine the relative physiological activity of certain members of the above series. The dose of any particular alcohol requisite to abolish the function of the frog's ventricle gave the measure of the activity of this alcohol. The following alcohols were taken: methylic, ethylic, propylic, butylic, amylic. The normal alcohols of the first three were selected; of the last two the isomerics were taken, viz. iso- and pseudo-butyl alcohol and isoamyl alcohol. In addition to these, isopropylic

alcohol was experimented with. The first three members alone of the series are consequently more strictly comparable if the object in view be the relation between molecular constitution and physiological activity. But from a practical point of view the whole series must be brought in, for, of the above, propylic, butylic, and amylic alcohols constitute, for the most part, the fusel oil which contaminates beer, wine and spirits."

The experiments of Ringer and Sainsbury were made with a Roy tonometer; the circulating fluid consisted "of a solution of desiccated bullock's blood in water, of about the concentration of normal blood; this was further diluted with two and a half times its volume of saline, 0.6 per cent. The ligature was applied as nearly as possible in the auriculo-ventricular groove. The heart, thus fed with the blood mixture, as a rule began soon to beat spontaneously. At definite intervals of time the drug was now added to the whole mass of the circulating fluid. The dosage was maintained uniform, and was such as previous experiment had shown to be sufficient to arrest the heart within an hour approximately. The restriction of time was for the purpose of eliminating the error due to the natural process of dying.

"The functions of the ventricle were in each case completely abolished by the end of the experiment, so that the ventricle neither beat spontaneously nor responded to electric excitation."

This is all that Ringer and Sainsbury state of their method, and is not as explicit as might be desired. They state that all of the alcohols examined arrested the heart in diastole. The following quantities (in minims) of the various alcohols were required to accomplish this effect:

Methyl, CH_4O.	Ethyl, C_2H_6O.	Propyl, C_3H_8O.	Butyl, $C_4H_{10}O$.	Amyl, $C_5H_{12}O$.
205.5	114	59.3	17	6.6

The same quantities expressed in *cubic centimetres* would appear as follows:

Methyl.	Ethyl.	Propyl.	Butyl.	Amyl.
12.1	6.73	3.5	1	0.4

Attention is called to the rapidly ascending ratio of activity as we pass from the lower and simpler alcohol to the higher and more complex members of the series; hence the first proposition is, *that activity increases with the complexity*; secondly, our attention is called to the fact that the numbers of the first three

members stand to each other very nearly in geometric progression. "The ethylic number is half the methylic, the propylic half the ethylic. The butylic and amylic numbers do not fit in the series, it is true; they do not, however, show any falling off in activity, but rather an increase." Continuing, Ringer and Sainsbury explain their physiological results by stating that a difference of CH_2, which is that by which each molecule in the series differs from its "preceding and succeeding terms, is capable of halving or doubling, as the case may be, the activity of the molecule; each CH_2 group may be said, in a way, to have its physiological equivalent. The proposition deduced, in short, is, that as long as the arrangement of the units is similar, as long as the lines of the structure remain the same, the addition or subtraction of each unit will carry with it a definite and constant difference. Mr. Perkin is said to have demonstrated as much for certain of the alcohol series, having shown that under certain definite conditions the addition of each CH_2 corresponds to a definite quantitative difference in the degree of polarization of light which the particular alcohol is capable of effecting." Three main conclusions are finally arrived at, viz:

1. The qualitative similarity of action of the different members of the alcoholic series.

2. The general quantitative relationship, viz. that as the complexity of the molecule increases, the physiological activity increases.

3. The probability of a further quantitative relationship, viz. that the constant chemical difference is corresponded to "by a constant physiological difference; that each additional CH_2 group increases the activity by a definite amount."

The work of Ringer and Sainsbury gives no evidence of any primary stimulation or increased frequence or force of the heart's contractions, on which point Dogiel attempted to confirm clinical experience; the results of the authors above rather confirm those of Prof. Martin, that the stimulation effect is *not* the result of direct action on the cardiac tissues.

The objections that can be urged against the work of Ringer and Sainsbury, as reported in the Practitioner for 1883, are first, lack of clearness in the statement of the method; second, employment of rather strong solutions of the alcohols. In the account of the method, the exact amount of the circulating

fluid is not stated, and we would not know how strong a percentage the circulating blood contained of alcohol if we were not told (p. 343) that diluting the poisoned circulation "with its own bulk (100 cc.) of saline, 0.6 per cent, caused quick recovery." Here we learn that the quantity of the solution of blood first used in each experiment was 100 cc.

The fact that dilution of the poisoned blood as stated above caused quicker recovery, seems to show that it is not the entire quantity of alcohol which flows through the heart in the whole experiment which ought to be taken into account, so much as the percentage strength which flows through the heart at any one time. As the quantity of the circulating fluid was 100 cc., we may arrive at the average percentage strength of the solutions of Ringer and Sainsbury by determining what per cent the various amounts of different alcohols which they found necessary to arrest the heart, are of the whole solution (100 cc.). Thus we find that solutions of the following strengths were used:

Methyl.	Ethyl.	Propyl.	Butyl.	Amyl.
12.1 per cent.	6.73 per cent.	3.5 per cent.	1 per cent.	.4 per cent.

Solutions as strong as these used upon isolated dogs' hearts would cause instantaneous death. Calculating the weight of the number of cc. of various alcohols used by Ringer and Sainsbury in grams (from the specific gravity tables of Landolt and Börnstein), the following number of grams are found to have been employed in each case:

Methyl alcohol,	. . . . .	9.6316 grams.
Ethyl "	. . . . .	5.3413 "
Propyl "	. . . . .	2.8693 "
Butyl "	. . . . .	.8242 "
Amyl "	. . . . .	.4148 "

To show the exceedingly large doses used by Ringer and Sainsbury, I suffix for sake of comparison the table of Dujardin Beaumetz and Audigé:

Alcohols.	Subcutaneous (pure).		Subcutaneous with Glycerine.		By Stomach with Glycerine.	
	Fatal Dose (grams).	Death in hours.	Fatal Dose in grams.	Death in hours.	Fatal Dose in grams.	Death in hours.
Ethyl C_2H_6O...	6–8.9	39–48	7.2	24	5.5–6.5	12–15
Propyl C_3H_8O..	4–4.5	..	3.0–3.6	24–36	3.0–3.4	12
Butyl $C_4H_{10}O$..	2–2.3	6–7	1.9	25	1.76	..
Amyl $C_5H_{12}O$..	1.8–2.3	2–7	1.3–1.63	..	1.4–1.55	13–0

Methyl alcohol, according to Dujardin Beaumetz and Audigé, is stronger than ethyl; 5 grams of pure CH_4O and 7 grams of diluted CH_4O being sufficient to produce death in 24 hours in a dog weighing one kilogram. By comparing the tables of Ringer and Sainsbury with those of Dujardin Beaumetz, it is evident that the former used solutions of alcohols on frogs which were far stronger than those required to kill dogs of weight of one kilogram in 24 hours.

It might be urged here that possibly the alcohols have a far less powerful effect on cold-blooded animals than on warm-blooded ones, which, perhaps, might explain the discrepancy.

To determine this I injected solutions of various alcohols of the strength used by Ringer and Sainsbury into the jugular veins of frogs, and found that without exception the heart had ceased beating in periods of time varying from 5 to 15 minutes.

This observation had been made long before on frogs with amyl alcohol, by Bergeron and L'Hôte (Compt. rend. CXI, No. 7; also Gaz. hebd. de méd. N. 35, p. 568). These investigators, in trying to show the detrimental effect of amyl alcohol used in the Erdmann-Uslar method of separation of morphine—a narcotic extract made by help of amyl alcohol—proved that considerable quantities of water shaken with amyl alcohol, in doses of a few drops, act as a rapid and fatal poison when injected subcutaneously in frogs, and similarly on guinea-pigs and rabbits when used in doses of a few cubic centimetres.

It is probably true that alcohol acts with less vigor on cold-blooded animals than on the warm-blooded; perhaps that conclusion might be deduced from the fact which I shall attempt to demonstrate later on, namely, that the physiological activity of alcohols increases with the temperature. Nevertheless it seems clear from my own observations on frogs, and those of Bergeron and L'Hôte, that the quantity of the various alcohols employed by Ringer and Sainsbury was probably too great to give accurate results.

In Volume III, p. 483, of the Studies from the Biological Laboratory of the Johns Hopkins University, is contained a very interesting report by the late Dr. J. R. Duggan on the "Influence of Alcohols on the Conversion of Starch by Diastase." The main idea suggested there is that the relation between

chemical constitution and physiological action of alcohols might be studied by the retardation produced by them in the conversion of starch by diastase; *i. e.* that the amount of any alcohol required to bring about a certain degree of retardation could be taken as a measure of its physiological strength. Duggan also made some experiments on the amounts required of each of the first three primary alcohols of the ethylic series to prevent, for a given time, the development of bacteria in a standard solution of beef peptones. The activity he found increased with the addition of each CH_2 group, with the exception of ethylic alcohol, which was found to cause a break in the series, being less strong than methylic. Dr. Duggan suggested that there might be in all alcohols a chemical property to which their action was due, which might be termed "alcoholicity," just as with acids we speak of "acidity," with bases of "alkalinity." As this "alcoholicity" could be counteracted by addition of small amounts of acid, he supposed that this hypothetical property was allied to alkalinity, and this led me to undertake three experiments on the isolated dog-heart, in which the alcohol was modified by minute additions (1–2 drops, according to his suggestions) of acetic or hydrochloric acid, with a view of ascertaining whether the effect of the alcohol would not be diminished thereby. The results, however, were negative, for blood solutions in which both alcohol and acid were present seemed to be more harmful to the heart than that which contained merely alcohol.

Dr. F. S. Lee, in the third volume of this Journal, has shown that alkalies in the circulation produce vascular constriction, in contradistinction to neutral salts and particular acids which produced dilatation. It seems certain, from observations of many, that the alcohols act as vascular dilators; their physiological action, therefore, is not analogous to that of alkalies.

This leads me to say a few words concerning the chemistry of the alcohols. According to differences in their molecular structure as manifested by differences in their conduct when oxidized, alcohols are divided into primary, secondary, and tertiary. In studying the relation between physiological activity and chemical constitution, it would be necessary that all alcohols used should belong to the same class.

The alcohols used in the series of experiments to be presently

described were all *fermentation* alcohols; they were all primary. These alcohols are normal—methyl, ethyl and propyl alcohol, isobutyl alcohol, and amyl alcohol of fermentation, which, as was shown by Pasteur, consists of two isomeric alcohols which both are primary.

The chemical differences between two primary representatives of the same alcohol are much less than between a secondary and a primary, or between a tertiary and a primary, or between a secondary and a tertiary; so that, as the normal butyl and amyl alcohols cannot be obtained except with difficulty, they are represented in my experiments by their primary isomers. The chemical relationships can be illustrated more clearly by representing the graphic or structural formulae of the first five members of the ethylic alcohol series.

I. Methylic, . . . $H\left(\begin{matrix}COH\\H_2\end{matrix}\right)$

II. Ethylic, ... $HC\left(\begin{matrix}COH\\H_2\end{matrix}\right)$

III. Propylic, . . . $HCC\left(\begin{matrix}COH\\H_2\end{matrix}\right)$

IV. Butylic, ... $HCCC\left(\begin{matrix}COH\\H_2\end{matrix}\right)$

V. Amylic, ... $HCCCC\left(\begin{matrix}COH\\H_2\end{matrix}\right)$

"It is evident that each formula in this series differs from its preceding one by the group CH_2; it is also evident that this addition does not imply derangement; each CH_2 thus interpolated enters into combinations precisely similar, and hence it must be apparent that whatever change in properties the addition of the CH_2 group effects in the first instance, a like change must be effected by the addition of the group CH_2 in the second instance, and so on. One is dealing here simply with a chain of like material and structure, but composed of more or fewer links. There is, however, one other point for mention, viz. that such a series, whilst it means quantitative difference, implies qualitative likeness.

"The secondary and tertiary alcohols cannot enter into such a series for comparison, for the additional CH_2 interpolated does not now enter into precisely similar rélations, but comes into

relation with the group $\overset{COH}{H_2}$, placed in brackets above, and so modifies the general structure.

"Finally, the isoprimary alcohols do not strictly fit into the above series; but the additional CH_2 groups do not modify the arrangement of the group $\overset{COH}{H_2}$, which is the more important group of the alcohol molecule; hence these primary isomerics, showing much smaller differences, may without much stretch be used for comparison."—(Ringer and Sainsbury.)

Influence of Alcohol on the Constituents of the Blood.

In considering the physiological action of alcohol, the question of its influence on the plasma and corpuscles of the blood arises. Not all of the alcohol mixed with the blood in circulation experiments enters the system and exerts its detrimental influence directly as alcohol; it is probable that a portion of it is used up or changed on coming in contact with the blood constituents. This seems to be confirmed by the observations of Buchheim (Archiv der Heilkunde, 1866, p. 124), which show that if alcohol is added to fresh blood outside of the body, it is so changed that even a short time afterwards very little of it can be regained. According to the same author, this is in some way a vital phenomenon of the blood, for blood which has been carefully kept from eighteen to twenty-four hours disposes of much less alcohol.

That alcohol is very quickly changed or gotten rid of somehow in the blood was shown by Professor Martin in experiments reported in a previous number of this Journal. Lallemand, Perrin and Duroy reported that alcohol accumulated in the blood, and more particularly in the brain and liver; according to J. W. Warren, their figures concerning the amount regained from the liver after administering large doses of alcohol are not convincing, for in the blood only 11 per cent was found, and in the brain not more than 1 per cent.

Anstie (Final Experiments, etc.) gave a small dog one ounce (198.9 grains) of brandy daily for ten days; the amount excreted on the tenth day was only 1.13 grains of alcohol. On the eleventh day the animal was given half an ounce of brandy, killed two hours later, and immediately cut up into small pieces and every

portion of it put into water for extraction. It appears that only twenty-four grains of alcohol were recovered, or not more than one-fourth of the amount which had been taken a few hours before. It seems plausible then that when alcohol reaches the blood directly or through the alimentary canal, a portion of it at least, and probably the largest, is changed to something else. Whatever this is we are not able to tell in the present state of our knowledge.

The direct action of alcohol on the blood constituents has been studied by Schmiedeberg (Petersburg Medic. Zeitschrift, 1868, B. 14, S. 92; also Virchow's Archiv, Vol. 51, p. 171). Here we are informed that blood mixed with alcohol does not give its oxygen up in the presence of a reducing agent as readily as the same blood in a pure state. As it is the rôle of haemoglobin to yield oxygen which has been taken up by the lungs to oxidizable blood and tissue constituents in the systemic circulation, the action of alcohol on blood just mentioned must be of great importance for processes in the living organism. A direct chemical influence of the alcohol, in addition to its action on heart and nerves, is therefore very probable, that is providing alcohol acts on the blood inside of the body in the same way it does outside.

Herrman (Archiv f. Anat. u. Physiolog., Reichert u. Dubois-Reymond, 1866, S. 27) describes the effect of the vapor of alcohol on blood thus: The rouleaux of corpuscles break asunder, the corpuscles become spheroidal, these minute spheres become paler and paler and finally disappear entirely, while the plasma colors intensely red and separates out crystals.

According to Marvaud (L'alcool, son action physiologique, etc. Paris, 1872, p. 14), alcohol produces a disturbance in the diasmotic relations between the corpuscles and the serum, whereby the nutrition of the former is interfered with.

The following interesting observations were made by Jaillet and Hayem, of which we find an abstract in Virchow and Hirsch, Jahrbücher d. Gesammt. Medizin, 1884, p. 369. In animals into which alcohol had rapidly penetrated from the stomach, these observers confirm an extensive alteration of the red blood corpuscles; scarcely one-third of them were intact, the remaining were partly of the mulberry type, with yellow

precipitates of haemoglobin in their interior. Still others were reduced in size and deprived of their haemoglobin entirely. On injecting alcohol directly into the blood, changed and destroyed corpuscles were found, but no mulberry forms. On introduction of gradual small doses the haematoblasts and leucocytes were strikingly increased, so too the coagulability of the blood. Blood gas analysis in animals under not quite intoxicating doses of alcohol, showed reduction in the respiratory capacity of blood corpuscles with considerable increase of CO_2 in the blood, which latter fact Jaillet partly brings into connection with the oxidation of the alcohol.

C. H. Schulz (Wirkung des Branntweins in der Trunksucht. Hufeland's Journal für pract. Heilkunde, 1841, April) had already described the separation of the haemoglobin from the corpuscles and the coagulation of the blood on addition of alcohol, in 1841.

Lallemand, Perrin and Duroy (Du rôle de l'alcool, etc., Paris, 1860) have observed in the blood of alcoholized animals, large quantities of oil globules floating on top after blood was drawn; these were, however, observable in the blood of the same animals after they had gotten over the effects of the alcohol. According to Baer (*Alcoholismus*), Magnus, Huss and others have observed the same in the blood from the heart and large veins of persons that died in intoxication. An interesting study of the effect of alcohol on febrile animals was made by Manassein. The corpuscles of the blood of such animals are very much diminished in size by the elevated temperature. This is not the case if the animals are given alcohol, when the corpuscles become larger the longer the intoxication lasts and the more the temperature is lowered by the alcohol.

All of these observations and experiments on the influence of alcohol on the constituents of the blood justify us in concluding that in circulation experiments on the isolated heart with alcoholized blood, all of the disturbances observed in the function of the organ are *not* due primarily and exclusively to the direct action of alcohol on the heart tissue, but that the action of the alcohol on the blood itself, whereby its nutritive and oxygenating functions are impaired, should enter into consideration.

Synopsis of the Method.

The method employed in this series of experiments for isolating the mammalian heart was that fully described in a previous number of this journal (Vol. II, p. 213, Plate XV), with a few changes of secondary importance, especially in the arteries that were connected to the outflow tube.

In my experiments both carotids were connected with a curved cannula, from the convexity of whose bend the outflow tube extended through the top of the warm chamber; here it ended in an outflow orifice, which emptied its blood into a funnel; from here the blood could be directed to any one of four large jars made of glass and converted into Mariotte's bottles. The same outflow tube was, by inserting a glass T tube at its lower end, connected with a mercury manometer which recorded the pulse on the paper of a kymograph. The height of the outflow orifice was so selected as to give a mean pressure of 130–150 mm. of mercury measured by the manometer. The circulating fluid was supplied from four large glass jars that were converted into Mariotte's flasks. Each flask could, by means of stop-cocks and connecting tubes, be connected at will with a cannula that was inserted into the vena cava superior. These stop-cocks were placed on the outside of the chamber. In the cannula, which was tied in the stump of the superior cava and only two inches from the right auricle, was inserted a thermometer which indicated the temperature of the blood as it entered the auricle. The supply bottles stood in large jars filled with warm water and acting as jackets. The vapor of the hot water from a trough in which the entire chamber rested continually surrounded everything inside, keeping the blood constantly at any desired temperature. If it was desired to lower the temperature, the Bunsen burners beneath the trough were extinguished and a large opening in the top of the case uncovered. If a high temperature was desired, the opening was closed and more burners lit beneath the trough. Thermometers hung up in various parts of the chamber rarely differed more than one degree C. from that in the vena cava cannula. The animals used were dogs. An attempt was made to get them approximately of the same weight.

The circulating nutrient liquid consisted of defibrinated fresh

calf's blood, and in some cases of defibrinated dog's blood. No difference in the effect of the same alcohol was observed on using the two kinds of blood. In two of the Mariotte's bottles were contained two litres of fresh defibrinated dog's or calf's blood, to which 500 cubic centimetres of a 0.75 solution of sodium chloride in distilled water had been added. In the remaining two bottles the same amount of blood was contained, but before it was mixed with the sodium chloride solution the alcohol was added to the latter in such quantity as to make $\frac{1}{8}$ per cent of the whole mixture. $\frac{1}{8}$ of one per cent was the amount of alcohol contained in all solutions in all experiments, with exception of those with amyl alcohol, where $\frac{1}{8}$ per cent was found, after repeated trials, to be too strong a solution to experiment with successfully, and solutions of $\frac{1}{10}$ per cent and $\frac{1}{20}$ per cent were employed, in addition to those containing $\frac{1}{8}$ per cent.

The same precautions were taken as in the experiments of Prof. Martin and L. T. Stevens, the outflow of any one of the four Mariotte's bottles in one minute not varying more than 3 per cent from that obtained from any of the other three. Generally three persons took part in the experiment. One attended to the working of the kymograph, a second had control of the turning on or turning off of pure or alcoholic blood and looking to the thermometers and recording the temperature, while a third was engaged in taking measurements of 30 seconds each at the outflow tube; the blood, collected in graduated vessels for measurement, was at once, as soon as noted, poured back into the funnel, from where it flowed into the receiving bottle.

Our way of determining the strength of any particular alcohol on the isolated dog's heart was not that of Ringer and Sainsbury, *i. e.* trying to establish the amount of alcohol required to kill the heart; as the exact moment of death of this organ is a point that can hardly be decided upon with accuracy. The method adopted here was to measure the amount of reduction that a particular alcohol effected in the quantity of blood pumped around by the heart in 30 seconds. When the amount of non-alcoholic blood pumped around by the heart was constant in four or five consecutive measurements, the alcohol-containing blood was turned on and measurements immediately undertaken, and the reduction produced in the amount pumped around in 30 seconds taken as

a measure of the effect of the alcohol. After the action of the alcohol had been clearly manifested, blood containing no alcohol was again turned on, when recovery from the effects of the drug as a rule occurred. Those experiments in which no good recovery occurred under pure blood were rejected, as the reduction in the work might have been due to causes independent of the action of the alcohol.

Results.

Explanation of the Tables.

In the tables given, only about one fifth of the successful experiments made are recorded, as it would be useless to print in detail four or five tables all illustrating the same facts. Only one experiment with each alcohol is printed in tabular form, except in the case of methyl alcohol, in which two are given; one commencing at a low, and the other at a high temperature. The tables are easily explained. There are four columns, headed from left to right thus: 1. Time; 2. Amount pumped in 30 seconds; 3. Temperature; 4. Kind of blood.

Taking the record of the experiment with methyl alcohol on April 20, 1886, we make the following observations: The heart was isolated completely at 2.15 p. m. In the measurements at 2.18, 2.20, 2.22, and 2.24 o'clock p. m., the heart pumped around 196 cc., 197 cc., 197 cc., and 196 cc., in 30 seconds respectively. This was while supplied with non-alcoholized blood, the temperature being 36° C. at the first three measurements, and sinking to 35° C. at the fourth. At 2.26 the alcoholized blood was turned on, and at 2.27 a measurement was taken which showed that the heart had only pumped around 186 cc. in 30 seconds, at a temperature of 35° C. As the previous measurements under pure blood gave 196 cc. in the same time and in the same temperature, we may say that at 35° C. a solution of $\frac{1}{8}$ per cent methyl alcohol will cause a fall in the pumped amount by isolated dog-heart in 30 seconds from 196 cc. to 186 cc., which is a reduction of about 5 per cent.

As the alcoholized blood continues to flow through the heart its action becomes more manifest. Thus at 2.29 p. m. the amount pumped around sank to 175 cc., and at 2.32 to 162 cc. At 2.33 pure blood was again turned on and the alcoholic blood turned

off; almost at once we notice recovery taking place, as shown by the increased amount of blood pumped out, which at 2.40 o'clock reached 194 cc. in 30 seconds. Then alcoholized blood was again turned on and pure blood turned off. At 2.43 the measurement shows a fall in the amount pumped around in 30 seconds to 174 cc. By this time the temperature had risen to 37° C. As the previous measurement under pure blood at 2.40 o'clock was 194 cc., we see that this second dose, $\frac{1}{8}$ per cent methyl alcohol in defibrinated calf's blood, caused a reduction of 20 cc. in the quantity of blood pumped by the left ventricle in half a minute. Similarly we observe, at 3.06 o'clock, a reduction from 170.5 to 138 cc., at 3.26 o'clock we observe a reduction in amount pumped around in 30 seconds of 50 cc. at 42°. By putting the observations together we obtain the following table. *One-fifth per cent solution of methyl alcohol in defibrinated calf's blood causes the following reductions (diminution in work) of the amount pumped around by an isolated dog-heart in* 30 *seconds:*

At 35° C.,	a fall	from	196 cc.	to	186 cc.,	or reduction	of	10 cc.
37° "	"		194 "		174 "	"		20 "
39° "	"		170 "		138 "	"		32 "
42° "	"		142 "		92 "	"		50 "

In a similar manner the average reduction in the work done by the heart under the other alcohols was determined, namely: (*a*) Noting the number of cubic centimetres pumped around just before the alcoholized blood was turned on; (*b*) noting the diminution in the number of cubic centimetres immediately after the blood containing the alcohol was turned on; (*c*) noting the temperature with each measurement.

With methyl alcohol four experiments were made in which the temperature was gradually increased from the beginning, and one experiment in which the temperature was gradually reduced from the onset. The same was the case with all of the remaining alcohols. In determining the general average reduction, expressed in cubic centimetres, which any particular alcohol would effect in the work done by the heart, only those four experiments were made use of in which the temperature was gradually raised from the onset, and that one in which the temperature was gradually reduced from the beginning of the

experiment was excluded, since it was thought more correct to determine the general average reduction (the reduction drawn from all the experiments with the same alcohol) only from experiments in which all conditions were alike. The average temperature at which the general reduction was in all cases calculated was 37° C., for reasons to be given later. The weights of the dogs experimented with, and the corresponding average reduction which the alcohol produced in the work done by the heart, are given in a separate table. In four experiments with *methyl* alcohol the reductions were:

April 20, 1886,	= 20.7	cc.
21, "	= 19.583	"
27, "	I = 18.583	"
27, "	II = 19.	"

By adding these four figures and dividing the sum by 4 one naturally obtains the mean or general average of the reductions observed in all the experiments under methyl alcohol, which is 19.46 cc.

The average weight of the animals used in these four experiments, as may be seen in the table, was 15,793 grams. So one may derive the following conclusion: $\frac{1}{8}$ per cent methyl alcohol in defibrinated blood will quickly, at 37° C., reduce the amount pumped around by the isolated heart of a dog weighing 15,793 grams in 30 seconds, by 19.46 cubic centimetres.

In the same manner the physiological effect of the remaining alcohols was determined. The following figures express the general average reductions of the first four alcohols:

Methyl, CH_4O, ...	19.46 cc.
Ethyl, C_2H_6O, ...	17.45
Propyl, C_3H_8O, ...	79.705
Butyl, $C_4H_{10}O$, ...	161.121

When we get to amyl alcohol we meet with difficulties. This member of the series proved too powerful to be used successfully in the same strength as the preceding members, namely $\frac{1}{8}$ per cent. It was found after repeated trials that solutions of amyl alcohol of this strength ($\frac{1}{4}$ per cent) might be used, if the time in which the measurements were taken was shortened to 15 seconds (these periods having been 30 seconds in all other experiments),

thus considerably abbreviating the time in which the heart was exposed to the poisonous influence of the drug. In two experiments with $\frac{1}{8}$ per cent of defibrinated calf's blood, in which measurements were taken of only 15 seconds' duration (May 28 and June 1, 1886), the general average reduction was found to be 161.16 cc. at 37° in fifteen seconds, equivalent, of course, to 322.32 cc. in half a minute.

In four experiments with amyl alcohol, however, in which solutions of $\frac{1}{10}$ per cent were employed and measurements taken for 30 seconds, the general average reduction was 161.508 cc., which is strikingly near the figure given above; so much so that one might believe the same effect could be obtained if one would double the strength of his solutions but halve the time of exposure, or halve the strength of the solutions and double the time of exposure. For in the experiments given, $\frac{1}{8}$ per cent amyl alcohol caused the same reduction in 15 seconds that $\frac{1}{10}$ per cent produced in 30 seconds. Whether such a relation as represented really exists I am not able as yet to say.

One experiment was undertaken with only $\frac{1}{20}$ per cent solution of amyl alcohol. Here the reduction observed in measurements lasting 30 seconds was 81.33 cc. This is just one-half (or very nearly so) the reduction effected by $\frac{1}{10}$ per cent solutions of this alcohol in 30 seconds, or one-half the reduction which $\frac{1}{8}$ per cent amyl alcohol caused in 15 seconds' measurement. So far we have the following average reductions observed under amyl alcohol, with solutions of varying strengths:

Amyl alcohol, solutions of.	Reduction in measurements of 15 seconds.
$\frac{1}{8}$ per cent,	161.16 cc.
	Measurements of 30 seconds.
$\frac{1}{10}$ "	161.508 cc.
$\frac{1}{20}$ "	81.33

The physiological effects of the various alcohols, according to our experiments, may be expressed as to the reductions they produce in the work during thirty seconds of the isolated hearts of dogs of nearly equal size, thus:

Methyl, CH_4O,	19.46 cc.
Ethyl, C_2H_6O,	17.45
Propyl, C_3H_8O,	79.705
Butyl, $C_4H_{10}O$,	161.121
Amyl, $C_5H_{12}O$,	322.32

We thus reach the following conclusions:

1. *That the physiological activity increases with the complexity of the molecule, with exception of the second member, viz. ethyl alcohol, which is weaker than methyl alcohol.*

With the exception of ethyl alcohol, the figures approach a geometric progression. Excluding ethyl alcohol, which is weaker than methyl, we have the following figures:

CH_4O.	C_2H_6O.	C_3H_8O.	$C_4H_{10}O$.	$C_5H_{12}O$.
19.46		79.705	161.121	322.32

Judging from this, the strength of propyl alcohol, C_3H_8O, is very nearly four times that of methyl. Butyl alcohol, $C_4H_{10}O$, is twice as powerful as propyl, and amyl alcohol twice as strong as butyl alcohol.

2. Another principal conclusion which is easily arrived at on examining the summary given at the end of each table, is that the *physiological activity increases as the temperature increases.*

In the experiment with $\frac{1}{5}$ per cent methyl alcohol on April 20th we may observe this fact in the summary:

At 35° C.	a reduction of	10 cc.	in the blood pumped out by the left ventricle in thirty seconds.
37	"	20	
39	"	32	
42	"	50	

On the other hand, in those experiments in which the temperature was gradually lowered from the onset (as in the following with methyl alcohol on April 23d), we have the following results:

At 40°	reduction of	31. cc.
39	"	26.
38	"	24.5
37	"	20.5
36	"	17.5

As the physiological activity of the various alcohols increases with the complexity of the molecule, so other properties increase, namely, the vapor density, the specific gravity, and the boiling point become higher. This becomes evident from the following table:

Name of alcohol.	Chemical formula.	Vapor density. $H_2=1$.	Specific gravity. $H_2O=1000$.	Boiling point.
Methyl	CH_4O	.16	.8142	55.1° C.
Ethyl	C_2H_6O	.23	.80625	78.3
Propyl	C_3H_8O	.30	.8198	98.0
Butyl	$C_4H_{10}O$	.37	.8242	117.0
Amyl	$C_5H_{12}O$	.44	.8296	135.0

The vapor density of these alcohols increases .7 for every increment in composition of CH_2. The boiling point increases on the average 19.6° for every increment of CH_2. There is also a constant increase of the specific gravity as the complexity of the molecule increases; this, however, only occurs in the second or third decimal point, and ethylic alcohol forms an exception to the statement.

Position of Ethylic Alcohol in the Series.

It is evident from my experiments and those of Dujardin Beaumetz and Audigé, and J. R. Duggan, that ethyl alcohol causes a break in the series, being less hurtful than methyl. Since ethylic alcohol is probably constantly present in minute quantities in the atmosphere, as fermentation is going on in many ways and places, and as dogs probably must frequently get some little ethyl alcohol in their food, this fact has been explained theoretically by supposing that an organism can establish a special degree of tolerance of one alcohol while retaining its sensitiveness to others.

A further explanation of the tolerance of ethylic alcohol by certain animals is furnished by a fact which is becoming more and more established, and that is that ethyl alcohol in minute quantities is a normal constituent of almost all animal tissues. One of the clearest investigations on this subject is published by A. Rajewski in Pflüger's Archiv (Bd. XI, p. 122, 1875), who obtained the iodoform reaction in the distillate of fresh liver, brain, and muscles of animals receiving no alcohol in their food. Rajewski concluded that these tissues either always contain certain quantities of ethyl alcohol, or that the iodoform test is not a sufficient proof of the existence of ethyl alcohol. C. Binz (Vorlesungen über Pharmakologie, Vol. II, p. 367) holds a similar view, according to which this test indicates alcohol in traces, but also carbohydrates, albumen, fibrin, casein and glue. According to E. Millon (Compt. rend., 1845, Vol. 4, p. 828), the iodoform reaction has been obtained with saliva of teetotalers. This reaction, as is perhaps well known, is carried out by adding a few drops of a solution of iodine in iodide of potassium to the solution to be examined; then just enough sodium hydroxide is added to make the yellow color disappear; then the mixture is

heated up to about 60°. On cooling, the solution becomes a yellowish tint, and the minutest trace of alcohol will cause the formation of iodoform, which can be recognized under the microscope by its hexagonal crystals. The reaction is the following:

$$8I + C_2H_6O + 6NaOH = 5H_2O + NaCHO_2 + 5NaI + CHI_3.$$

Although this reaction is not reliable in testing for alcohol in living tissues, it has been satisfactorily shown that these do contain alcohol. One of the chief workers in this field is J. Bechamp (Paris). In the Compt. rendus, T. LXXXIX, p. 573 (Sur la presence de l'alcool dans les tissues animaux pendant la vie et après la mort), Bechamp states that he extracted 0.8 gram of alcohol from a piece of meat weighing 6 pounds, that he has found alcohol in sheep's liver immediately after death; also in human brain. This investigator has also found alcohol to be a normal constituent of fresh milk (Jour. Chem. Soc., Vol. 26, p. 763). He has developed a theory concerning fermentations *intra vitam* which is based on what are termed by him the *microzymes*. These are described by him as being the physiologically active parts of cells of animal tissues; they are minute granular masses that have the power of inducing fermentation like other cellular ferments, but differ in quality and energy of action according to the part of the organism from which they are taken. He supposes them to be the centre of all chemical changes of living and dead tissues (Bechamp, Archiv de Physiol. Normal et Pathol.), and to belong to the animal organism as integral parts without having to get into it from the air or other medium; they are, even in perfect health, capable of causing all those processes *intra vitam* which are commonly known as fermentations, and *post mortem* all those metamorphoses which are comprised under the phenomena of decomposition. All soluble ferments are products or secretions of microzymes, and are called zymases (A. Bechamp, Archiv de Physiol. 1882, p. 28). Diastase, trypsin, and pepsin are termed hordeozymase, pancreazymase, and gastrozymase.

It is impossible here to enter upon the method of J. and A. Bechamp, of isolating these microzymes, which is given in Compt. rend. LXXXI, pp. 226–229. My intention merely is to point out a theory according to which the existence of ethyl

alcohol in living tissues might be explained, and also the tolerance of certain animals toward this alcohol.

Cause of the Diminution in the Work of the Isolated Heart under Alcohol.

In a previous number of this Journal, Vol. 2, p. 487, Professor H. N. Martin has considered those alterations in the physical condition of the heart which impede its action and diminish its work. The question whether the alcohol acted on the nerves and ganglia, the blood-vessels or on the muscular tissue of the heart was discussed, and the conclusion reached that the main effect of the drug is exerted upon the cardiac muscular tissue. It is possible that the blood-vessels of the coronary system are also acted upon to a certain extent. The most obvious phenomenon, as pointed out by Professor Martin, is the great dilation of the isolated heart poisoned by ethyl alcohol, accompanied by an imperfect systole, so that the ventricular cavity is not obliterated at the end of the systolic contraction, and the less so the longer the alcohol has been administered. This incomplete systole is compensated for by a more extensive diastole as long as the heart can swell. As soon as the pericardium prevents this excessive expansion in diastole, the difference between diastolic and systolic capacity becomes less and less, and the heart pumps around less blood. An interesting matter in this connection is a fact which I have observed in several cases in which the heart was expanded and swollen to a great extent under alcohol, which is, that by placing a stethoscope directly on the organ one can hear plainly so-called regurgitant murmurs. These murmurs are undoubtedly on both sides of the heart. Perhaps they are caused by the swelling and expanding of the heart excentrically, whereby the fibrous rings to which the valves are attached are so enlarged that the valves are no longer large enough to cover them, and there are tricuspid and mitral insufficiency and regurgitation. In all my experiments small hemorrhages into the cardiac tissue were observed; these were very considerable with the higher alcohols. It was thought that they were the direct result of the action of the alcohols; but by injecting considerable quantities of alcohol into the jugular veins of dogs, I could in no instance produce them on the heart.

The reasons why all averages were calculated at 37° were that it was found that this temperature could be most easily maintained, and also that the averages could not well have been drawn at a higher temperature on account of the increase in poisonous action, particularly of amyl alcohol, which in the strength used invariably produced rapid death of the heart above 38°. No change in the pulse rate due to the alcohol used was noticed in any of my experiments.

The writer wishes to express his indebtedness to Professor H. Newell Martin for advice and many useful suggestions.

Experiment with one-fifth per cent CH_4O methyl alcohol, April 20, 1886. Dog under morphine and curare. Defibrinated calf's blood used. Heart isolated at 2.15 p. m.; remained in good condition 1 hr. 17 min.

Time.	Amount pumped around.	In 30 sec.	At temp.	Kind of blood.
2.18	196 cc.	...	36°	Pure.
2.20	197	"	36	"
2.22	197	"	36	"
2.24	196	"	35	"
2.26	...	...	...	CH_4O ⅓ %.
2.27	186	"	35	"
2.29	175	"	35.5	"
2.32	162	"	36	"
2.33	...	...	...	Pure.
2.34	174	"	36	"
2.36	185	"	36.5	"
2.38	194	"	36.5	"
2.40	194	"	37	"
2.42	...	...	...	CH_4O ⅓ %.
2.43	174	"	37	"
2.45	160	"	37	"
2.47	148	"	37.5	"
2.50	102	"	38	"
2.51	...	...	...	Pure.
2.53	136	"	38.5	"
2.54	158.5	"	38.5	"
2.56	169.75	"	39	"
2.58	168.75	"	39	"
3.01	170	"	39	"
3.03	...	...	...	CH_4O ⅓ %.
3.06	138	"	39	"
3.09	130	"	39.5	"
3.11	114	"	40	"

Time.	Amount pumped around.	In 30 sec.	At temp.	Kind of blood.
3.12	...	...	...	Pure.
3.14	120	"	40	"
3.16	132	"	40	"
3.18	141.5	"	40.5	"
3.20	142	"	41	"
3.25	...	...	...	CH_4O ⅛ %.
3.26	92	"	42	"
3.28	70	"	42	"
3.30	40	"	42	"
	Stopped pumping.			
3.31	...	...	...	Pure.
3.32	Began pumping around again, but very feebly. Temperature toward end too high; probably caused death.			

Action of CH_4O ⅛ per cent increases with the temperature. Thus:

At 35° centigrade we observe a fall from 196 cc. to 186 cc., or 10 cc. in 30 seconds.

At 37° centigrade we observe a fall from 194 cc. to 174 cc., or 20 cc. in 30 seconds.

At 39° centigrade we observe a fall from 170 cc. to 138 cc., or 32 cc. in 30 seconds.

At 42° centigrade we observe a fall from 142 cc. to 92 cc., or 50 cc. in 30 seconds.

This gives an average of 20.7 cc. reduction of amount pumped around under ⅛ per cent CH_4O at temperature 37° C. in 30 seconds, measured one minute after CH_4O ⅛ per cent blood was turned on, and calculated from the average of the measurements at 35° C., 37° and 39°.

Experiment April 23d, 1886, methyl alcohol ⅛ per cent in defibrinated calf's blood. Dog under morphine and curare. Heart isolated 2.40 p. m.; under experimentation for 1 hour and 20 minutes.

Time.	Amount pumped.	In 30 sec.	Temp.	Kind of blood.
2.43	128 cc.	...	40°	Pure.
2.44	129	"	40.5	"
2.45	131.5	"	40.5	"
2.46	132	"	40	"
2.47	...	...	...	Methyl alcohol ⅛ %.
2.48	101	"	40	"
2.49	75	"	40	"
2.50	48	"	40	"
2.51	20	"	39.5	"

Time.	Amount pumped.	In 30 sec.	Temp.	Kind of blood.
2.52	...	...	...	Pure blood.
2.53	45	"	39.5	"
2.54	76	"	39.5	"
2.55	105.5	"	39.5	"
2.56	128.5	"	39	"
2.57	132	"	39	"
2.59	...	...	...	Methyl alc. ⅓ %.
3.00	106	"	39	"
3.02	82	"	39	"
3.03	58	"	38.5	"
3.05	32	"	38.5	"
3.07	...	...	...	Pure blood.
3.08	60	"	38.5	"
3.09	81.5	"	38.5	"
3.10	105	"	38.5	"
3.11	128	"	38	"
3.12	129	"	38	"
3.14	129.5	"	38	"
3.15	...	...	...	Methyl alc. ⅓ %.
3.16	105	"	38	"
3.17	81	"	38	"
3.18	55	"	37.5	"
3.19	30	"	37.5	"
3.20	...	...	...	Pure blood.
3.22	70	"	37.5	"
3.25	124	"	37.5	"
3.26	128	"	37	"
3.28	128.5	"	37	"
3.29	...	...	...	Methyl alc. ⅓ %.
3.30	108	"	37	"
3.32	65	"	36.5	"
3.34	24	"	36.5	"
3.35	...	...	36.5	Pure blood.
3.37	66.5	"	36.5	"
3.39	102	"	36.5	"
3.41	124	"	36	"
3.43	127	"	36	"
3.45	127.5	"	36	"
3.46	...	...	...	Methyl alc. ⅓ %.
3.47	110	"	36	"
3.49	95	"	36	"
3.51	78	"	36	"
3.53	60 heart irregular.	"	36	"
3.55	...	...	...	Pure blood.
3.56	60 more irregular.	...	...	"
3.58	20	...	...	"
4.00	Heart did not recover under pure blood.			

Experiment April 23, 1886. Results with methyl alcohol $\frac{1}{8}$ per cent.

In this experiment the temperature was taken high (40°) at the beginning of the experiment, and gradually lowered as it progressed. In other experiments the effect of the alcohol increased as the temperature increased; in this case it decreases as the temperature decreases; in both cases showing dependence upon the temperature. The effects of $\frac{1}{8}$ per cent methyl alcohol as shown by the table are:

At 40°	a fall	from	132	cc.	to	101	cc.,	or reduction of 31 cc.
39	"		132	"		106	"	" 26
38	"		129.5	"		105	"	" 24.5
37	"		128.5	"		108	"	" 20.5
36	"		127.5	"		110	"	" 17.5

Heart stopped in diastole, very much swollen and covered with minute ecchymoses.

The average taken from above measurements at 36°, 37° and 38° is 20.83 cc. reduction in amount pumped around in 30 seconds at 37°.

Average reduction of all (4) experiments with $\frac{1}{8}$ per cent methyl alcohol (excluding the one on April 23, 1886):

April 20, 1886,	20.7 cc.	reduction	at 37°.
April 21, 1886,	19.583	"	"
April 27, 1886, I =	18.583	"	"
April 27, 1886, II =	19	"	"

4)77.866(19.46 cc. = total average reduction of amount pumped around in 30 seconds at 37° C. by isolated dog's heart (calculated from results of four experiments).

Experiment March 4, 1886. Ethyl alcohol $\frac{1}{8}$ per cent in defibrinated dog's blood. Dog under morphine and curare. Heart isolated 1.24 p. m.; remained good for 1 hour and 34 minutes.

Time.	Amount pumped.	In 30 seconds.	Temp.	Kind of blood.
1.26	136 cc.	...	35°	Pure blood.
1.28	138	"	35	"
1.30	138.5	"	35	"

Time.	Amount pumped.	In 30 seconds.	Temp.	Kind of blood.
1.32	...	...	...	Ethyl alcohol ½ %.
1.33	130	"	35	"
1.34	121.5	"	35.5	"
1.35	112	"	35.5	"
1.36	100	"	35.5	"
1.37	...	...	...	Pure blood.
1.38	110.5	"	35.5	"
1.39	118	"	35.5	"
1.41	136	"	36	"
1.43	137.5	"	36	"
1.44	...	...	...	Ethyl alcohol ½ %.
1.45	128	"	36	"
1.47	101	"	36	"
1.49	76	"	36.5	"
1.51	64.5	"	36.5	"
1.52	...	...	...	Pure blood.
1.53	78	"	36.5	"
1.54	90	"	36.5	"
1.56	112	"	36.5	"
1.58	126	"	36.5	"
2.00	135	37	37	"
2.01	136	"	37	"
2.03	...	...	...	Ethyl alcohol ½ %.
2.04	115	"	37	"
2.05	95.5	"	37	"
2.06	76	"	37.5	Ethyl alcohol ½ %.
2.07	58	"	37.5	"
2.08	...	...	...	Pure blood.
2.10	78	"	37.5	"
2.12	101	"	37.5	"
2.14	124.5	"	38	"
2.16	134	"	38	"
2.18	135	"	38	"
2.20	...	...	...	Ethyl alcohol ½ %.
2.21	110	"	38	"
2.23	91.5	"	38	"
2.25	68	"	38.5	"
2.27	30.5	"	38.5	"
2.28	...	...	...	Pure blood.
2.29	61	"	38.5	"
2.32	104	"	38.5	"
2.35	130.5	"	38.5	"
2.37	133	"	39	"
2.39	134.5	"	39	"
2.42	...	...	...	Ethyl alcohol ½ %.
2.43	103	"	39	"
2.44	75	"	39	"

Time.	Amount pumped.	In 30 seconds.	Temp.	Kind of blood.
2.45	...	...	...	Pure blood.
2.47	98	"	39.5	"
2.49	126	"	39.5	"
2.51	132	"	39.5	"
2.53	134	"	39.5	"
2.54	...	...	...	Ethyl alcohol ½ %.
2.55	100	"	39.5	"
2.56	68 cc. heart-beat irregular.	"	40	"
2.57	...	"	40	Pure blood.
2.58	72 cc. kymograph tracing feeble.	"	40	"
2.59	64 cc.	"	40	"
3.00	40 cc., heart dying.	"	40.5	"

Results:

At 35° a fall from 138.5 cc. to 130 cc., or reduction of 8.5 cc.

36	"	137.5	"	123	"	14.5
37	"	136	"	115	"	21
38	"	135	"	110	"	25
39	"	134.5	"	103	"	31.5
39.5	"	134	"	100	"	34

Average taken from the measurements at the temperature of 36°, 37°, 38°, is 20.16 cc. reduction at 37° in amount pumped around in 30 seconds.

Experiment No. II, May 20th, 1886. Propyl alcohol ½ per cent in defibrinated calf's blood. Isolation completed 3.18 p. m. Heart under experimentation for 46 minutes.

Time.	Amount pumped.	In 30 seconds.	Temp.	Kind of blood.
3.20	168 cc.	...	35.5°	Pure blood.
3.21	170	"	35.5	"
3.22	170	"	35.5	"
3.23 30 sec.	...	...	36	Propyl alcohol ½ %.
3.24 15	105	"	36	"
3.25	74	"	36	"
3.26	42.5	"	36	"
3.26 30	...	...	36	Pure blood.
3.27 30	72	"	36.5	"
3.28 15	100	"	36.5	"
3.29	134.5	"	36.5	"
3.30	164	"	36.5	"
3.30 45	166	"	36.5	"
3.31 30	168	"	36.5	"
3.32 30	168	"	37	"

Time.	Amount pumped.	In 30 seconds.	Temp.	Kind of blood.
3.33	...	...	37	Propyl alcohol ⅓ %.
3.33 50	85	"	37	"
3.34 45	50.5	"	37	"
3.35 30	25.5	"	37	"
3.36	...	...	37	Pure blood.
3.37	60.5	"	37	"
3.38	82	"	37	"
3.39	120	"	37.5	"
3.40	148	"	37.5	"
3.41	161	"	37.5	"
3.42	163	"	37.5	"
3.43	164	"	37.5	"
3.43 30	...	...	38	Propyl alcohol ⅓ %.
3.44 20	75.5	"	38	"
3.45 38	45.5	"	38	"
3.46 30	25	"	38	"
3.47	...	...	38	Pure blood.
3.48	49.5	"	38	"
3.49	78	"	38.5	"
3.50	110	"	38.5	"
3.51	160	"	38.5	"
3.51 45	159	"	38.5	"
3.52 15	160	"	38.5	"
3.53	...	...	39	Propyl alcohol ⅓ %.
3.53 48	60.5	"	39	"
3.54 30	20	"	39	"
3.55	Stopped.	"	39	"
3.55 30	...	...	39	Pure blood.
3.56 15	80.5	"	39.5	"
3.57	58	"	39.5	"
3.58	64	"	39.5	"
3.59	91	"	39.5	"
3.59 45	108	"	39.5	"
4. 30	145	"	40	"
4.01	148	"	40	"
4.02	149	"	40	"
4.03	150	"	40	"
4.03 30	...	...	...	Propyl alcohol ⅓ %.
4.04 15	80	"	40	"
4.05	Stopped.	"	40	"
4.06	...	...	40.5	Pure blood.
	Heart did not recover under pure blood.		40.5	"

Experiment No. II, May 20, 1886. Propyl alcohol ⅓ per cent. Results:

At 36° a fall from 170 cc. to 105 cc., or reduction of 65 cc. in amount pumped around in 30 seconds.

At 37° a fall from 168 cc. to 85 cc., or reduction of 83 cc. in amount pumped around in 30 seconds.

At 38° a fall from 164 cc. to 75.5 cc., or reduction of 88 cc. in amount pumped around in 30 seconds.

At 39° a fall from 160 cc. to 60.5 cc., or reduction of 99.5 cc. in amount pumped around in 30 seconds.

At 40° a fall from 150 cc. to 30 cc., or reduction of 120 cc. in amount pumped around in 30 seconds.

The average reduction calculated from the first three measurements is 78.83 cc. in 30 seconds at 37°.

Results of four experiments with $\frac{1}{8}$ per cent propyl alcohol:

1. May 18, 1886, No. I, 79. cc. reduction at 37° in 30 seconds.
2. " 18, " " II, 80.83 " 37 "
3. " 20, " " I, 80.16 " 37 "
4. " 20, " " II, 78.83 " 37 "

4)318.82(79.705.

Total average reduction effected by $\frac{1}{8}$ per cent propyl alcohol in defibrinated calf's blood (result of four experiments) = 79.705 cc. at 37° in amount pumped around by the isolated dog-heart in 30 seconds.

Experiment No. I, May 24, 1886. Isobutyl alcohol $C_4H_{10}O$ $\frac{1}{8}$ per cent in defibrinated calf's blood. Dog under morphine and curare. Heart isolated 2.8 minutes p. m.; remained in good condition for 30 minutes.

Time.	Amount pumped.	In 30 seconds.	Temp.	Kind of blood.
2.10	196 cc.	...	35.5°	Pure.
2.10 30 sec.	197	"	35.5	"
2.11	198	"	35.5	"
2.11 30	197	"	36	"
2.12	...	...	...	Butyl alc. $\frac{1}{8}$ %.
2.12 45	45	"	36	"
2.13 30	5	"	36	"
2.13 40	Stopped.	"	36	"
2.14	...	...	...	Pure blood.
2.14 45	24.5	"	36.5	"
2.15 30	78	"	36.5	"
2.16 15	122	"	36.5	"
2.17 10	158.5	"	36.5	"
2.18	179	"	36.5	"

Time.	Amount pumped.	In 30 seconds.	Temp.	Kind of blood.
2.18 40	195	"	36.5	Pure blood.
2.19 15	194	"	37	"
2.19 45	...	...	...	Butyl alc. $\frac{1}{5}$ %.
2.20 30	32.5	"	37	"
2.21	3	"	37	"
2.21 10	Stopped.	"	37	"
2.21 45	...	...	37.5	Pure blood.
2.22 30	41.5	"	37.5	"
2.23 30	85	"	37.5	"
2.24 30	140.5	"	37.5	"
2.25 30	186	"	37.5	"
2.26 30	194.5	"	38	"
2.27 15	192.5	"	38	"
2.27 45	...	...	...	Butyl alc. $\frac{1}{5}$ %.
2.28 30	21	"	38	"
2.29	Stopped.	"	38	"
2.29 40	...	...	38	Pure blood
2.30 30	85	"	38	"
2.31 30	68.5	"	38.5	"
2.32 30	97.5	"	38.5	"
2.33 30	129	"	38.5	"
2.35	186	"	38.5	"
2.36	186	"	39	"
2.36 15	...	...	39	Butyl alc. $\frac{1}{5}$ %.
2.37	A few drops, about 2 cc.	"	39	"
2.37 10	Then stopped.	"	39	"
2.37 40	...	...	...	Pure.
2.38	Heart did not begin to pump around again under pure blood.			

Results of $\frac{1}{5}$ per cent solution of primary isobutyl alcohol in defibrinated calf's blood on isolated dog-heart:

At 36° a fall from 197 cc. to 45 cc., or reduction of 152 cc. in 30 seconds.

At 37° a fall from 194 cc. to 32.5 cc., or reduction of 161.5 cc. in 30 seconds.

At 38° a fall from 192.5 cc. to 21 cc., or reduction of 171.5 cc. in 30 seconds.

At 39° a fall from 186 cc. to 2 cc., or reduction of 184 cc. (practically 186) in 30 seconds.

After the last measurement at 39° the heart did not recover.

Average, 161.66 cc. at 37° in 30 minutes (taken from first three measurements).

In the experiments with butyl and amyl alcohols measurements were taken as often as possible. Four large graduated

glasses were placed in a row next to the outflow tube, which was bent like a hook. As soon as thirty seconds were counted, during which blood was measured into one graduate with the 31st second, the glass (bent end) of the outflow tube was hooked over another empty graduate, while the first was noted and emptied back into supply bottle, and so on with the 3d and 4th graduate. By the time the fourth was used, the first graduate was again ready for measuring.

May 24, 1886. I. Experiment.—Heart and lungs covered with ecchymoses the size of a large pea. Lungs very oedematous. Left ventricle at end of experiment ceased beating before the right.

Experiment No. I, June 3, 1886. Amyl alcohol $\frac{1}{10}$ per cent in defibrinated calf's blood. Dog under morphine and curare. Isolation completed 1.40 p. m.; heart lived 26 min. 30 sec.

Time.	Amount pumped.	In 30 seconds.	Temp.	Kind of blood.
1.45	193 cc.	...	35.5°	Pure.
1.46	194	"	35.5	"
1.46 30	194	"	35.5	"
1.47 15	193.5	"	36	"
1.48	...	...	36	Amyl alcohol $\frac{1}{10}$ %.
1.48 45	43	"	36	"
1.49 15	Stopped.	...	36.5	"
1.49 45	...	...	36.5	Pure blood.
1.50 30	83	"	36.5	"
1.51	111	"	36.5	"
1.52	182.5	"	36.5	"
1.52 30	184	"	36.5	"
1.53 15	184.5	"	37	"
1.53 45	...	...	37	Amyl alcohol $\frac{1}{10}$ %.
1.54 30	25	"	37	"
1.55	Stopped.	"	37.5	"
1.55 30	...	...	37.5	Pure blood.
1.56 15	20	"	37.5	"
1.57	94.5	"	37.5	"
1.57 45	124	"	37.5	"
1.58 30	181	"	37.5	"
1.59 15	182	"	37.5	"
2.	182.5	"	38	"
2. 30	182	"	38	"
2.01 15	...	...	38	Amyl alcohol $\frac{1}{10}$ %.
2.02 30	A few drops, about 1 cc.	"	38	"
2.02 40	Stopped.	...	38	"

Time.	Amount pumped.	In 30 seconds.	Temp.	Kind of blood.
2.03	...	...	38	Pure blood.
2.03 45	10	"	38.5	"
2.04 30	24	"	38.5	"
2.05 15	49	"	38.5	"
2.06	98	"	38.5	"
2.06 35	120	"	38.5	"
2.07	124	"	39	"
2.07 30	174	"	39	"
2.08	180	"	39	"
2.08 30	180	"	39	"
2.09 30	...	"	39	Amyl alcohol $\frac{1}{10}$ %.
2.10	Stopped.	"	39.5	"
2.10 45	"	...	39.5	Pure blood.
2.11 30	Began again, but very irregular and weak.			"

Notes.—Heart and lungs covered with large ecchymoses. Measurements were taken very nearly once in 30 seconds.

Results.—At 36° a fall from 193.5 cc. to 43 cc., or reduction of 150.5 cc.

At 37° a fall from 184.5 cc. to 25 cc., or reduction of 159.5 cc.

At 38° a fall from 182 cc. to 1 cc., or reduction of 181 cc.

This gives an average of 163.366 reduction at 37° in the amount pumped around in 30 seconds.

The last measurement under amyl alcohol taken at 2 o'clock 9 minutes 30 seconds, is not reliable, because, as the drug produced a reduction of 181 cc. at 38°, it would certainly from what we know have had a greater effect than 180 cc. at 39.5°. Moreover, the heart did not in the latter case make a recovery.

Results of Experiments with Amyl Alcohol.

$\frac{1}{8}$ per cent $C_5H_{12}O$.—Reduction in amount pumped around by isolated dog-heart in 15 seconds at 37°, effected by $\frac{1}{8}$ per cent solution of amyl alcohol:

May 28, 1886, 161.66 cc. June 1, 1886, 160.66 cc. Average, 161.16 cc. at 37° in 15 seconds.

$\frac{1}{10}$ per cent $C_5H_{12}O$.—Reductions under $\frac{1}{10}$ per cent amyl alcohol:

May 27, 1886, 159 cc. June 3, 1886 (I), 163.366 cc. June 3, 1886 (II), 163.83 cc. June 4, 1886, 159.83. Average, 161.508 cc. reduction in 30 seconds at 37°.

If we may judge from two experiments with $\frac{1}{8}$ per cent $C_5H_{12}O$, and compare them with the general result of four experi-

ments with $\frac{1}{10}$ per cent $C_5H_{12}O$, it is evident that $\frac{1}{3}$ per cent will cause the same reduction in 15 seconds that $\frac{1}{10}$ did in 30 seconds.

$\frac{1}{20}$ per cent $C_5H_{12}O$.—Reduction observed in one experiment in which $\frac{1}{20}$ per cent amyl alcohol was employed, 81.33 cc. reduction in 30 seconds at 37°.

Average of one experiment with $\frac{1}{10}$ amyl alcohol which was started at a high temperature and gradually lowered, June 4, 1886, 158.83 cc. reduction at 37° in amount pumped around in 30 seconds.

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ON THE VENTRICULAR EPITHELIUM OF THE FROG'S BRAIN.

By A. C. WIGHTMAN, B. A., Fellow by Courtesy of the Johns Hopkins University. With Plate XXII.

In the July number of the Johns Hopkins University Circulars for 1888 there appeared a short abstract, "The Ventricular Epithelium of the Frog's Brain." The following paper is a more detailed account of the research described in that abstract. Before proceeding to describe the methods and results of my work, a brief account of the results reached by previous investigators is desirable.

In 1844, Adolphe Hannover (1),[1] who was the first to study the frog's brain with a microscope, stated that all the ventricles of the brain are lined by conical, ciliated cells, from whose deeper ends long fibers extend toward the periphery. He considered the cells, not as epithelial, but as true nervous cells.

Alphonse Blattmann (2), in 1850, describes the walls of the ventricles as being lined with ciliated epithelium.

Jeffries Wyman (3), in 1852, says the brain cavities are lined with ciliated epithelial cells.

In 1864, E. Reissner (4), in his excellent description of the central nervous system of the frog, says, in reference to the epithelium of the central canal of the spinal cord—that the cells are cylindrical, spherical or spindle-shaped; that it is uncertain whether they are ciliated or not; that from the tips of the cells, the bases being turned towards the cavity of the ventricle, long processes run out into the grey substance. The thickness of the epithelial layer he gives as 0.036μ; an isolated cell is in length from 0.024μ–0.036μ; in breadth, 0.005μ–0.008μ; the nuclei have diameters from 0.006μ–0.012μ. Frequently the nucleus possesses no nucleoli, but is filled with fine granules. There is a definite membrane lining the bases of the epithelial cells. As regards the brain, he states that in the *sinus rhomboideus* the cells are much like those of the central canal of the spinal cord, and that

[1] These numbers refer to the "Literature" at the end of the paper.

the variations which occur are mostly in size, seldom in form. In several places, as in the roofs of the third and fourth ventricles, the long epithelial cells pass into flat cells; from the inner ends of these cells processes can be traced far into the brain. In chromic acid preparations he described a clotted seam on the bases of the cells, from which projected clusters of fine hairs. Ciliary movement could be detected in fresh specimens, by employment of pigment or a little chalk. Reissner used vitreous humor to moisten the epithelial surface in order to observe the movements of the cilia.

Schöne (5), 1865, denies the presence of an epithelial lining to the central canal of the spinal cord.

In 1869, Ludwig Stieda (6) published his work on the nervous system of vertebrates; he says, in speaking of the epithelium, that the central canal of the spinal cord is lined by epithelial cells which are cone-shaped, the bases being turned toward the cavity. The cells measure at their bases, in breadth .004μ, in length about .04μ. The nuclei are large, round or oblong, and .002μ in length. From the cells bordering the anterior and posterior (ventral and dorsal respectively) surfaces of the canal, fine fibers or processes run off, which processes become attached to similar processes of the *pia mater*. The cells bordering the lateral regions of the canal have processes shorter and finer. The brain cavities, like the central canal, are lined by a single layer of cylindrical epithelium. A long process runs out from the tip of each cell. At certain regions of the brain the epithelium changes in character. Wherever *pia mater* alone covers the ventricle—as in the *ventriculus communis loborum hemisphaericum, the third ventricle*, under the *cerebellum* and so forth—the epithelium lining that surface of the *pia* facing the ventricular cavity becomes flattened.

In another publication in 1875, Stieda (9) describes the corresponding epithelium of the Axolotl. In the spinal cord of this animal the cells are not regularly arranged around the central canal, but are in groups. The cells are of two kinds: one spindle-shaped, with a long neck, the other cone-shaped. The nuclei of these two kinds of cells lying at different levels, those of the spindle cells lying farther from the canal, give the appearance of a double row of cells, whereas there is only a single layer. The

cells vary so in size that no satisfactory measurements can be given. The nuclei measure .012μ–.018μ. The processes from these cells connect with the *pia mater*. In the fourth ventricle the cells are regularly arranged, are cone or spindle-shaped, with their bases towards the ventricle; here the cells vary in length from .021μ–.024μ; in breadth are about .015μ. The relation of cone and spindle cells is the same as in the central canal, the long necks of the spindle cells being inserted between the cone cells. The epithelium of the other ventricles resembles that of the fourth ventricle.

Stieda, in describing the central nervous system of the tortoise (10), says that in the spinal cord he could not establish such connection between epithelial processes and the *pia mater* as existed in the frog. The cells are small, ill-defined, and possess nuclei of from .007μ–.009μ in size. The structure of the cell and the presence of cilia could not be made out. The majority of the cells lining the ventricles are cylindrical; cone and spindle cells are also found. Cells vary in the different portions of the brain; all grades of transitional forms can be seen. The choroid plexuses of the brain are all constructed alike; they consist of a combination of *pia mater* and epithelial cells. The *pia* becomes much folded, and the folds are lined with cells which are continuous with those of the ventricles of the brain. The blood-vessels run in these folds. The cells are not ciliated.

Mierzejewsky (7) published his paper in 1872. He says that the epithelial cells of the ventricles and of the central canal form a continuous layer. They are cylindrical or flattened in shape. All are ciliated. The cells of the ventricular epithelium send out long processes. In the central canal, the cells on the ventral side are much larger and possess much longer processes than those on the dorsal side. There is, however, no sharp division line between them. The same is true of the ventricular epithelium of the brain; the cells on the ventral surfaces being much larger and possessing longer processes than those on the dorsal.

Karabanowitsch (8) in 1872 declares that all the cells lining the central canal are ciliated, and that the long processes, penetrating into the cord, lose themselves in the connective tissue.

Hoffmann (11) in 1878 agrees with Stieda and Reissner in their descriptions of the epithelium of spinal cord and brain.

Mason (12) also says that in the frog the epithelium of the ventricles of the brain and of the central canal of the cord consists of conical ciliated cells. From these cells processes run off, to become continuous with the *substantia reticularis*.

It is maintained by Gierke, in 1883 (13) and again in 1885 (14), that the epithelial cells of the ventricles and of the central canal are connected by their processes with processes from glia cells.

Obersteiner (16) says that the tendency of modern research is to show that the epithelium of the ventricles of the brain and of the central canal of the cord is of a nervous nature. Klaussner has shown that the processes from the epithelial cells of the spinal cord of *Proteus anguineus* can be followed, some into the posterior root, some into the anterior commissure. All the cells are ciliated and possess large oval nuclei having nucleoli. It is probable that the epithelium lining the medulla is most intimately related to the nervous elements. All the ganglion cells of the central organ are derived from the epithelial cells, though the transition from one form to the other is not clear. In *Amocoetes*, according to Herms, large ganglion cells arise within the ventricular epithelium by the quick growth of certain cells. These cells then sink into the brain substance, to become ganglion cells. Freud found that the processes from the epithelial cells and nerve fibers stained exactly alike with gold.

In my own work the following method was adopted to extract the brain and spinal cord of the frog from the cranium and vertebral column uninjured. The frog used was the *Rana catesbiana*, the common bull-frog. The animal was killed with ether or chloroform; after removing the skin from the dorsal surface, the muscles of the vertebra were stripped off up to the base of the skull. With a pair of scissors the vertebral column was severed just anterior to the sacral vertebra. Holding the spinous process of one of the vertebra with the forceps, it is easy to introduce a pair of scissors into the neural canal and to successively clip the neural arches of all the vertebrae, working from posterior to anterior, lifting off at the same time the separated portion. In this way the whole of the dorsal half of the vertebral column and of the cranium can be taken off, leaving

the brain and spinal cord absolutely uninjured. Then after cutting the roots of all the cerebro-spinal nerves, the central nervous system can be easily lifted out.

The brain and spinal cord so obtained were transferred to some one of the usual hardening agents, as Müller's fluid, potassium bichromate, corrosive sublimate, nitric acid, or osmic acid.

For the purpose of staining, hæmatoxylin, Gaule's quadruple method (hæmatoxylin, nigrosin, saffranin, eosin), Weigert's hæmatoxylin method, Golgi's silver nitrate method, and gold chloride were used. The method which proved most satisfactory was to place the brain and spinal cord in five per cent nitric acid for two hours, wash out well in running water for half an hour, transfer to 30 per cent alcohol for three hours, then to 50 per cent for two hours, 70 per cent for one hour, 90–95 per cent indefinitely—two days is enough. After hardening sufficiently, the brain and spinal cord were well washed in water and then soaked, for half an hour at least, with a 2 per cent solution of potassium bichromate, after that they were quickly washed in distilled water and transferred to a solution of Böhmer's hæmatoxylin. In this solution of hæmatoxylin the specimen was kept for two to three days, heating the solution each day for one hour up to 38° C. It was next well washed in water, dehydrated by alcohol, embedded, and sections cut with the microtome and mounted in the usual manner. This method, a combination of those recommended by C. S. Minot and A. B. Lee, yields excellent stains of the epithelial lining of the brain. In the stained sections any surplus color can easily be removed by acidulated alcohol.

For the preparation of teased specimens, Dogiel's method (15) was followed with excellent results. The brain and spinal cord were removed from the neural cavity, and the ventricles and central canal were laid open so as to give free access to the reagent; then the whole was placed in a one per cent solution of osmic acid for 18–24 hours, and next, after being thoroughly washed with distilled water, were placed aside to macerate in a little water for one week. The specimen was finally transferred to a mixture of one half glycerine, one half water for a day, then into pure glycerine. Small portions of the brain were placed, with a drop of glycerine, under a cover glass supported by a bit

of paper to prevent undue pressure. By tapping, or by giving a gentle sucking action to the cover glass by alternately pressing and relieving, the cells can readily be separated.

Distribution of the epithelium.—The epithelium of the frog's brain and spinal cord forms a continuous lining to all the cavities of the central nervous system. It is everywhere a single layer thick. Though forming a continuous lining, yet the character and size of the cells vary considerably. In the *lateral ventricles* (Plate XXII, Figs. 1, 2, 3), at the middle region of the right and left surfaces of the cavity, the cells are regular in shape and size and clearly in a single layer; at the dorsal and ventral angles of the cavity, these cells become slightly larger, more crowded, and less regularly arranged. From the tip of each epithelial cell a tail-like process can be seen running into the brain substance, where it becomes lost.

In the *third ventricle*, at the dorsal (except on the roof of the ventricle) and ventral portions of the cavity, the cells are typical epithelial cells (Plate XXII, Figs. 4, 5, 6); in the middle region, however, these cells are larger, more elongated, apparently two layers deep, and from their tips much stronger processes penetrate the brain substance. Those cells lining the internal surface of the *pia mater* forming the roof of the ventricle are, on the contrary, small, cuboidal, and apparently tailless. The columnar and conical cells of the lateral walls of the ventricle pass gradually into these cuboidal cells. (Plate XXII, Fig. 4.)

Stieda (6) and Mierzejewsky (7) have also noticed this change, see extracts given above. In the region of the *optic lobes* (Fig. 7), the cells present, also, different appearances at different parts; those lining the cavity of the optic lobes being small, regular, and not particularly noticeable; from them small tail processes run into the brain substance; those cells lining the Aqueduct of Sylvius are more than twice as large, are much more crowded, especially at the ventral angle of the cavity, and have extremely long and noticeable processes (Figs. 7 and 8). In the *fourth ventricle* (Figs. 9, 10, 11) the cells lining the floor are comparatively large and possess long and strong processes, the cells of the roof of the ventricle are smaller and their processes are not so conspicuous. This apparently is the general rule, the epithe-

lial cells on the ventral surfaces of the ventricles are larger than those on the dorsal, see Mierzejewsky (7) quoted above. It is certainly true for the frog (Figs. 4, 7, 8, 9, 10, 11).

The relation of the epithelial cells to the brain cells.—The most cursory examination of a series of sections from the brain of the frog will reveal the fact that there is a definite relation between brain cells and the epithelial lining. Passing from the fore-brain backwards and beginning our examination with a section taken from the region of the *cerebral hemispheres* (Fig. 1), it will be seen that the cells of the brain are arranged, not promiscuously, but in groups and layers concentric to the epithelium; the processes from these latter cells giving the brain a striated appearance. More posteriorly in the *third ventricle*, this concentric arrangement is still more striking (Fig. 4). Here not only are the brain cells arranged concentric to the epithelium, but there is a special point in the lateral portion of the epithelial lining which seems to be a center around which the brain cells are grouped. In Fig. 4 this point is shown on the lateral walls at the level where the epithelium appears folded; Fig. 5 also shows it, but cells here are larger, more numerous and apparently in several layers, compare this with Fig. 6 taken from the same side more ventrally. At this central region, also, the processes are longer, larger and more numerous. Passing still more posteriorly in the *third ventricle*, the same arrangement is seen.

In the region of the *optic lobes* (Fig. 7) the relation of brain cells to epithelial cells is the same. The concentric grouping is striking; close to the epithelial lining of the optic lobe cavity is a layer of brain cells, just beyond a layer made up of fibers from cells and processes from the epithelium; next a layer of brain cells not so closely packed as the first layer; then another fibrous layer, then another cell layer, another fibrous, and so on, the layers becoming less and less definite until the sharp distinction between cell layer and fibrous is lost and the cells and fibers become promiscuously mixed. In the *Aqueduct of Sylvius* the epithelial cells are of the same size and shape as those at the central region of the third ventricle (Figs. 7 and 8). The processes from these cells can easily be traced into the brain substance for a distance nine or ten times longer than the cells.

The ventral angle of the Aqueduct of Sylvius is most interesting (Fig. 8). The epithelial cells can be very distinctly made out and their processes followed for an astonishing distance. Close beneath the epithelial layer lie little packets or groups of cells. The clusters contain varying numbers of them. Each cell of the cluster sends out two processes, one goes to an epithelial cell and becomes united with it, the other penetrates the brain substance and becomes lost. Apparently the clusters and groups of cells are derived from the close-lying epithelial layer. The size and shape of the nuclei of both classes of cells, their behavior towards staining reagents, and the direct connection with one another of the cluster and epithelial cells, indicate that these cells of the clusters so closely related to brain cells, are probably intermediate between nerve and epithelial cells. The tail processes of the epithelial cells lining the ventricles of the frog's brain are therefore to be regarded as connections or the remnants of connections between epithelial and nerve cells.[1]

In the *fourth ventricle* (Figs. 9, 10, 11) the same general arrangement is found. The floor of the ventricle is lined with large epithelial cells, each sending out into the brain substance a long, strong process (Fig. 2). In Fig. 9 the cerebellum and medulla are outlined; the cells of the cerebellum in such a section show no concentric arrangement. Fig. 11 will show the size, shape and arrangement of the cells on the under surface of the cerebellum; they are smaller, more regularly set, and do not possess such pronounced processes as those lining the rest of the ventricles of the brain. The concentric arrangement is clearly shown in the medulla (Fig. 9).

Structure and connections of the epithelial cells.—Leaving now the examination of stained specimens and passing to a study of teased preparations, it was found, as Stieda has already shown for the epithelium of the axolotl (9), that there are several kinds of cells which vary much in size and shape. However, every form of gradation between each kind can readily be found. The cells of the epithelium may be conveniently described as consisting of two typical kinds, a columnar and a spindle-like form,

[1] See Obersteiner (16) quoted above, page 264.

with intermediate shapes. On surface view the epithelium shows closely packed cells (the columnar-shaped cells) presenting a polygonal area, while the spindle cells and their varieties are represented by oblong spots or thick short lines scattered between the cells.

The columnar cells (Figs. 17, 18 and 22) consist of a stout, more or less regular body; from the basal end of each there projects into the ventricular cavity a closely set cluster of cilia. Each cilium bears at its base, where it joins the cell, an enlargement. This row of enlargements is the cause of the so-called "basal seam" when seen under a low power of the microscope. The protoplasm of the cell can be followed directly into the cilia. The other end of the cell tapers, more or less suddenly, into a long, comparatively stout protoplasmic process.

The other class of cells, the spindle (Figs. 12, 15, 22, 23), mainly consists of an oval or oblong body. From this central portion the body tapers into two processes, one going to the ventricle, where it expands again at the surface into a small enlargement. On this little head is borne a cluster of cilia, each having its basal swelling; the other process runs from the body of the cell and forms a long tail which penetrates far into the brain substance.

Cells are frequently found possessed of an extremely long and thin ventricular process with no expanded tip (Figs. 18 and 25); others are seen whose ventricular processes are scarcely at all contracted (Figs. 15, 20, 22); then there are found cells of a true cone shape, with no ventricular process whatever (Figs. 18, 19, 21, 23, 27); finally we easily pass to the other distinct class, the columnar (Figs. 17, 18, 22). In Fig. 28 is shown a bit of teased epithelium, in which the several classes of cells are easily seen.

An interesting modification of the spindle cells is shown in Figs. 13 and 14. Here the central body also sends out two processes, the one running into the brain substance, the other extending towards the ventricle. This latter in its course expands so as to become spoon-shaped, fitting nicely in between the cone-shaped or columnar cells. On the external ventricular rim of the spoon-shaped process is seen the row of cilia.

As Stieda (9) showed that in the epithelium of the spinal cord of the axolotl the cells were in a single row, the appearance of a double row of cells being due to the nuclei being at different

levels, so here the appearance of several layers of cells, as seen in Figs. 4, 5, 6, 7 and 8, is due to the different relative positions of the nuclei belonging to the several classes of cells described above. (See Figs. 15, 18 and 23.) The epithelium of the ventricles of the frog's brain is everywhere a single layer of cells.

As to the size of the cells, Reissner's (4) measurements are approximately correct: length .024μ–.036μ, breadth .005μ–.008μ, length of the nucleus .006μ–.012μ.

The tail processes extending from the deeper end of each cell into the brain substance deserve especial attention. It will be seen on referring to the historical portion of this paper that the majority of investigators believe that these tail processes are connected with the pia mater. The results of carefully teased preparations, however, do not support this opinion. The processes vary much in size and length. They are small and delicate in the lateral hemispheres, large and long in the third ventricle, small and delicate in the cavity of the optic lobes, strong and well developed in the aqueduct of Sylvius and the fourth ventricle. Successfully teased preparations show (1) the processes *are branched*, (2) *one at least of the branches ends in a brain cell.* (Figs. 16, 19, 23, 26 and 27.) Though the tail processes are branched, yet a process in connection with two or more brain cells has not been found.

Observations on the living cells.—The brain and spinal cord of a frog were carefully removed and placed in a glass trough under normal salt solution. The cerebral hemispheres were cut open by a longitudinal slit through the dorsal angles of each hemisphere, so as to expose the epithelium of the lateral ventricles; the same was done for the ventricles of the optic lobes, the aqueduct of Sylvius, the fourth ventricle and the central canal of the spinal cord; a continuous ciliated passage from the lateral ventricles down to the central canal was thus obtained. With the microscope the cilia could now be seen in active motion. If now, with a pipette, a drop of water containing finely ground carmine be placed in the cavity of any of the ventricles, a most interesting sight is obtained. Some of the carmine particles, settling in the water, are caught on the cilia. This affords an excellent means of watching the movements; the little particles of carmine

are vigorously lashed to and fro. At a temperature of 15° C. the following measurements were made:

No. 1. With a comparatively large carmine particle, a cilium in the third ventricle beat 100 times per minute.

No. 2. In the same field of the microscope and close to No. 1, a cilium bearing a piece of carmine about one-half as large as that of No. 1 beat 120 per minute.

No. 3. In fourth ventricle a cilium beat 108 per minute, the carmine particle being larger than that of No. 2.

No. 4. Fourth ventricle cilium beat 144 per minute, carmine smaller than No. 2.

No. 5. Fourth ventricle 138 per minute, carmine about the same size as that of No. 2.

The cilia of the cells of the spinal canal also beat at the rate of from 100 to 200 strokes per minute. No such thing as a wave of ciliary motion could be observed passing along the ventricles, but all over the microscopic field cilia could be seen beating independently, some slowly, some quickly, frequently side by side at different rates. Each beat consisted of a quick, vigorous stroke and a comparatively slow recovery. By carefully watching it was seen that the vigorous strokes were directed always posteriorly; consequently, as indicated by floating carmine particles, a current of water was driven from the fore-brain backwards.

In no case was there evidence of the existence of limiting membranes, either external or internal, to the epithelial lining of the ventricles (Fig. 28). The cells sometimes show a tendency to adhere by their bases, the ventricular ends, but this may be due to the presence of an adhesive substance, or to the interlocking of the hardened cilia.

The brain and spinal cord of the tadpole of the same species of frog were also examined, employing the same methods. The results were identical with those given above.

Conclusions. 1. The epithelial layer of the frog's brain and spinal cord forms a continuous lining to the central nervous system. It is everywhere a single layer thick.

2. The epithelium of the ventricles forms a central zone of cells, about which the brain cells are concentrically arranged.

3. The cells of the epithelium and of the brain are connected by processes which extend from the tips of the former.

4. The epithelial layer consists of cells of several varieties, the columnar, the spindle and intermediate forms. All are ciliated.

Before closing, I desire to express my thanks to Professor Martin for valuable suggestions, and to Dr. Brooks, Dr. Howell, and Dr. Donaldson for many kindnesses extended during the progress of my work.

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This bibliography is not a complete list of the work done on the central nervous system of the frog, but it contains merely the most important papers which refer to the structure of the ependyma of the frog's brain.

EXPLANATION OF PLATE XXII.

FIG. 1.—Cross section. Cerebral hemisphere, showing lateral ventricle. Nitric acid and hæmatoxylin. Zeiss' camera, Obj. A.

FIG. 2.—Upper or dorsal angle of the ventricle, from Fig. 1. Zeiss' D.

FIG. 3.—Lower angle of ventricle. Zeiss' D.

FIG. 4.—Section across anterior portion of the third ventricle. General view. Zeiss' camera, Obj. A. Treatment same as Fig. 1.

FIG. 5.—Middle portion of left ventricular wall, from Fig. 4. Zeiss' camera, D.

FIG. 6.—Lower part of same side. Zeiss' camera, D.

FIG. 7.—Section from the optic lobes, showing optic lobe cavity and the aqueduct of Sylvius. Zeiss' camera, Obj. A.

FIG. 8.—Ventral angle of aqueduct of Sylvius, from Fig. 7. Zeiss' camera, Obj. D. Nitric acid and hæmatoxylin. Shows the increase in size and the connections of the epithelial cells very clearly.

FIG. 9.—Section across the cerebellum and fourth ventricle. Nitric acid and hæmatoxylin. Zeiss' camera, Obj. A. The details of the structure of the cerebellum and medulla have not been drawn.

FIG. 10.—Epithelium at ventral angle of the fourth ventricle. Zeiss' camera, Obj. D of Fig. 9.

FIG. 11.—Epithelium of the cerebellum wall of Fig. 9. Zeiss' camera, Obj. D. Gaule's quadruple method.

FIG. 19.—Isolated group of cells from lateral ventricles. Shows ciliated epithelial cell, branched process, and union with brain cell. Dogiel's method. 4, D. Zeiss.

FIGS. 15 and 18.—Groups of epithelial cells with processes.

FIGS. 12, 20, 21 and 25.—Isolated cells. Third ventricle.

FIG. 28.—Piece of the wall of the third ventricle, shows the different classes of cells.

FIGS. 13 and 14.—Cells from third ventricle, with spoon-shaped ventricular processes.

FIG. 24.—Isolated cell with branched process. Lateral ventricle.

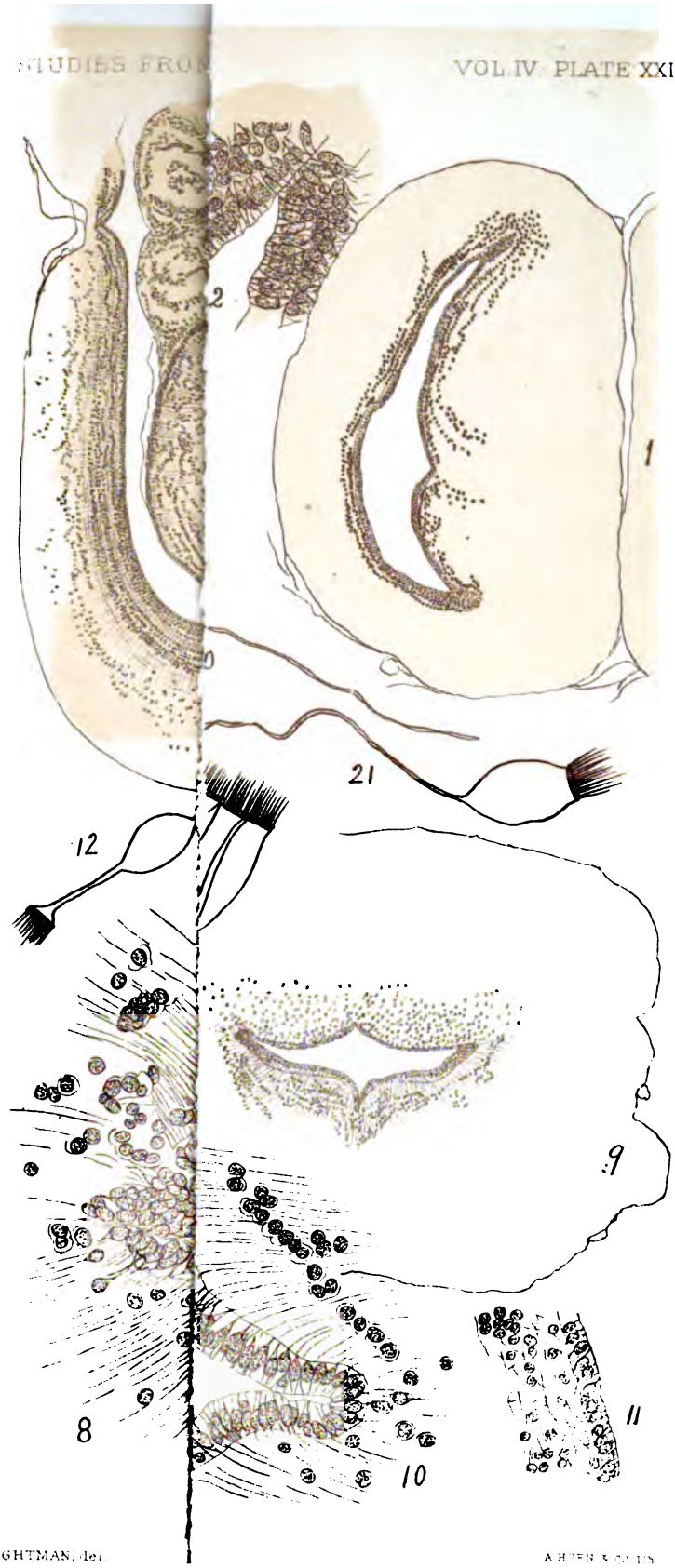
FIGS. 12, 17 and 22.—Cells of the columnar and spindle varieties. Third ventricle and aqueduct of Sylvius.

FIG. 23.—Cells from fourth ventricle. Spindle cells and a cone cell are shown; the process from the cone cell unites with a multipolar brain cell. Zeiss' 4, D.

FIG. 27.—Third ventricle, ciliated cell sends out the process which joins a brain cell. Zeiss' camera, D.

FIG. 16.—Optic lobe cavity. Shows connection of epithelium cell to brain cell.

FIG. 26.—Third ventricle. Isolated cell with branched process joining a brain cell.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ON THE TEMPERATURE LIMITS OF THE VITALITY OF THE MAMMALIAN HEART.

By H. NEWELL MARTIN, M. A., M. D., D. Sc., F. R. S., and E. C. APPLEGARTH, B. A., Fellow of the Johns Hopkins University. With Plates XXIII, XXIV, XXV.

Some years ago one of us[1] published the results of a research showing that between the limits 27° C. and 41° C., the heart of the dog, when isolated from all other organs but the lungs, beats quicker the higher its temperature, so that by heating or cooling the blood supplied to the isolated organ through the superior vena cava, the pulse rate could be controlled.

These earlier experiments made it clear for the range of temperatures above stated, that the tendency to beat quicker at a higher temperature was a fundamental property of the heart, and that the cardiac muscle of the dog agreed in the dependence of its rhythm upon temperature changes with the histologically very different muscle tissue of the frog's heart. A question which then presented itself was, What are the limits of temperature within which the heart of a mammal will beat at all? It also seemed of interest to ascertain whether mammalian cardiac muscle agreed with amphibian in having an optimum temperature at which its rate of beat was quickest, beyond which any increase would slow the rhythm, without necessarily killing the heart. That there was some such optimum for the dog's heart was rendered probable by the fact that in some of the earlier experiments[2] it appeared that heating the organ above 42° C. slowed its beat.

Attempts made three or four years ago to solve these problems were futile, because near the liminal temperatures, high or low, the heart beat with so little force that its death seemed rather due to deficient circulation through the coronary capillaries than to direct influence of heat or cold on the cardiac muscular or nervous tissues. Our problem was, therefore, to keep the cardiac

[1] H. N. M. Phil. Trans. Roy. Soc. 1883, Pt. II, p. 663.

[2] Phil. Trans. 1883, Pt. II, table on p. 681.

vessels well supplied with blood whether the heart beat feebly or strongly, and at the same time to vary its temperature at will. The first requirement it seemed we might attain by connecting the aortic stump of the isolated heart with a Mariotte flask filled with blood and kept at a constant level above the organ. Under such circumstances a constant pressure would be maintained in the coronary arteries, quite independent of the force of the heart's beat. If this could be done and the heart kept alive, it would be easy to add arrangements for varying the temperature. A preliminary experiment or two having showed that the plan was feasible, we arranged our apparatus essentially as indicated diagrammaticly in Plate XXIII.

The heart was isolated in the manner described in previous numbers of this Journal, and throughout the experiment was contained in a large moist chamber, *A*; part of the roof and of one end of this are indicated at *a* and *a'* in the figure. This chamber has no floor; it sits in a shallow iron trough containing water, which, during an experiment, is heated by Bunsen's burners placed under the trough so as to keep it and the chamber warm, and the air within the chamber saturated with moisture. This air is (so far as necessary occasional opening the doors of the chamber will permit) kept at a temperature ranging between 38° and 40° C. The chamber used was that described in the first number of the fourth volume of these Studies (p. 41), and figured in Plate V accompanying that number. Most accessories had, however, to be changed when the experiments described in this article were undertaken; the Mariotte flasks, for instance, were placed outside the chamber instead of inside it; and a new form of aortic cannula had to be devised.

The animal (cats were used in all cases) was rendered insensible by inhalation of ether or by subcutaneous injection of paraldehyde, and then given curare in some cases, in others not. Paraldehyde in doses of about 5 cc. we found on the whole the most satisfactory. Tracheotomy was next performed (as a preliminary to opening the thorax), and the wind-pipe connected with the apparatus for artificial respiration. The thorax having been opened, and most vessels of the systemic circulation tied essentially as described previously,[1] the glass cannula shown in

[1] Stud. from Biol. Lab., Vol. IV, p. 36.

Plate XXIII was tied into the distal end of the aortic arch. This cannula[1] has three side-pieces; through the one farthest from the heart it is supplied with defibrinated blood from the Mariotte flasks through the rubber tube *p*; through the next the most of this blood is carried off through the tube *q* and poured into a funnel; the height of this funnel above the heart of course determines the pressure in the aortic stump. A thermometer was tied into the third branch of the cannula, as shown in the diagram. All branches of the aortic arch except the coronaries were of course closed. The blood supplied to the aortic stump could thus escape only through *q* into the funnel shown in the plate, or through the circuit *q'*, indicated by dotted lines, and consisting of the coronary vessels. The blood taking the coronary circuit, on reaching the right auricle proceeded to the corresponding ventricle, and from it through the lungs to the left auricle. This blood (that taking circuit *q'*) was therefore the only blood entering the cavities of the heart or passing through the lungs, unless there were some inefficiency of the aortic semilunar valves. That the cavities of the heart were not distended with more blood, we found not to influence the normal character of its beat, which continued in many cases forcibly and rhythmically for three or four hours. The beat of the frog's heart, as well known, is much promoted by moderately high intracardiac pressure; that this is not, at least to so great an extent, the case in mammals seems easily explicable. The frog's heart, having no capillaries, depends for its nourishment or the washing out of its wastes, on the forcing of liquid under pressure into the spongy network of the ventricle, while in the mammal the nourishment of the heart depends on pressure in the aortic arch, from which the coronary system is supplied.

The side tube *q* is designed to get rid of the difficulty of insufficient aëration of the blood; were it not present, the only flow from the aorta would be through the coronary system, and the flow would be so slow that it would be impossible to renew the blood in the cannula fast enough to keep it from using up its own oxygen and becoming very venous, and unfitted to keep the heart at work. But by a free outflow through *q*, the

[1] The cannula was modified in the later experiments, as it was found more easy to manipulate when made in two pieces (p. 280).

blood in the cannula is quickly changed, and, moreover, so rapid a bubbling of air through the supplying Mariotte flask secured, that the flask takes the place of a lung and supplies arterial blood. The blood flowing from *q* into the funnel is collected in a beaker and poured every minute or two into the receiving Mariotte bottle, until the supplying bottle is nearly empty and the other full, then the stop-cocks are reversed and the receiving becomes the supplying bottle, and *vice versa*.

The preceding sketch of the general plan of an experiment will probably be sufficient for most readers. In practice many unforeseen difficulties had to be overcome, which may be of interest to those desiring to further investigate the subject. A primary difficulty, which it took us long to meet successfully, was, that in a progressive heating or cooling experiment, the blood during its circuit changed its temperature so much that we could not, on interchanging the supplying and feeding Mariotte bottles, get a continuous series of observations. This was overcome by an arrangement which poured water of desired temperature into the trough containing the Mariotte bottles; thence it flowed through wide tubes forming water-jackets for the tubes *p* and *q*, Pl. XXIII, and then into a water-jacket surrounding the funnel receiving from *q*, and emptying into the tank containing the beakers, from which the circulating blood was returned to one of the Mariotte bottles. Another difficulty which we soon discovered was that the temperature of the heart was by no means always that of the blood supplied to it; so a thermometer inserted through the *precava*, and with its bulb reaching into the right ventricle, was used to indicate the actual temperature of the heart, instead of the thermometer in the cannula. In the tables which follow, it is the record of the thermometer placed inside the right heart which is given.

The moist chamber (*A*, Plate XXIV) had on its top the two Mariotte flasks securely fastened in a tin box, *B*, serving as a water-jacket. One side of *B* is of glass and turned towards the assistant to enable him to see readily when a flask is nearly empty, so that by reversing the stop-cocks he may convert it from the supplying into the receiving flask. The flasks each hold about 700 cub. cent. From near the bottom of each passes the outflow tube, which is conveyed through the spouts *h* of the tin box into the warm chamber, and is continued by the rubber tube *T* (the left one is for the greater part omitted in the plate), to an aperture in the end of the warm chamber through which it passes to one leg of the Y-piece *q*, Plate XXV. Each upper leg, *j*, of the Y-piece has a stop-cock on it (*bs*), so that the supply may be taken from either Mariotte flask at pleasure.

Each Mariotte flask is closed air-tight by a rubber cork pierced by three glass tubes (Plate XXIV); one of these, *o*, is connected by a Y-piece with the funnel *P*, from which it gets blood when acting as receiving flask; a second, *n*, serves to let air out as blood enters the flask; both *n* and *o* are of course closed when the flask is acting as supply bottle to the heart. The third tube, *m*, reaches to near the bottom of the flask, and through it air bubbles in when the flask is acting as supply bottle. From its upper end passes the rubber tube, seen again

at *m* in Fig. 1, Plate XXV, where only the tube of one side is drawn; of course in actual use there must be a like tube attached to the other Mariotte flask. The long rubber tube *m* passes through the cork of a tightly closed test-tube, *Tb*, containing a little water. Another tube, *at*, passes to near the bottom of the test-tube and is open. When the Mariotte flask is at work all the air entering it must first bubble through the water in the test-tube, which, hanging outside the chest and the water-jacket, is easily observed, and indicates how fast the blood from the flask is flowing, or if the bubbling stop, that something has gone wrong and the supply bottle has ceased to act as a Mariotte flask. It is not necessary to describe in detail the stop-cocks which lie on all the tubes so that they can be in a moment closed or opened when the functions of the two flasks are to be reversed.

All the system of blood-carrying tubes is inclosed in a set of water tubes continuous with the spouts *hh* of *B*, and having warm or cold water from *B* flowing steadily through them during an experiment. Over those ends of each of the spouts which open into the warm chamber is fitted thin rubber tubing 1½ inches in diameter; these tubes surround the blood-conveying tubes *TT*, and finally end (Fig. 2, Plate XXV) in *J* and *J*, which open into a funnel-shaped tin water-jacket. Inside this jacket the Y-piece *q* (Fig. 1, Plate XXV) is placed, and its upper limbs *jj* pass through *JJ* to join *TT*. On the sides of the tin jacket are openings through which pass the handles (*bdr*, Fig. 2) of the stop-cocks *bs bs* (Fig. 1).

The lower limb *Q*, Fig. 2, Plate XXV, of the tin jacket surrounds *q*, Fig. 1 (which conveys the blood again into the warm chamber) and is continued over *C*. This tube (*C*, Plate XXIV) leads to the aortic cannula *a*, and up to about six inches from that point is jacketed by a wide rubber tube. At this point the jacketing tube is securely closed by a rubber stopper, through which a glass cannula, *a*, continues the blood-carrying system to the aortic stump. The reason for stopping the jacketing for a short distance at this point is to facilitate insertion of the cannula.

So far, then, it will be seen that water supplied to *B*, Plate XXIV, will flow out through the wide tubing surrounding *TT* through the jacket *JJ* (Plate XXV) to the tube around *C*, keeping the blood under essentially the same temperature conditions during its whole journey from the supply bottle to the heart. From near the rubber cork closing the jacket of *C* the water finds its exit through a side branch, a wide rubber tube surrounding *e*, and so to the wide glass funnel *f*, Plate XXIV. This funnel has a short stem into which is accurately fitted the stem of the blood-receiving funnel (*R*) by means of rubber tubing. From *f* the water issues through the outflow piece through the jacketing hose *l* into the tin kettle *y* (Plate XXV) containing the beakers for catching the blood; and finally into the waste bucket through the tube *Wst*.

The only parts of the blood-conveying system, then, which are not jacketed are the funnel *P*, the tubes *oo* leading from it to the Mariotte bottles, and the cannulas near the heart with a few inches of adjacent tubing. With the exception of the funnel *P*, however, most of these, including the innominate and aortic cannulas, were packed in a layer of cotton wool, thus checking any rapid loss or gain of heat.

All the water-jackets, or rather the whole jacket, for the system is but one, are supplied directly from the water pipe of the room. This pipe by means of a T piece is connected with an air chamber whose capacity is 18 gals., and which is capable of standing a high pressure to the square inch. From the bottom of this chamber a delivery pipe, *W*, Fig. 1, Plate XXV, runs to terminate in the stop-cock *D*, which stands in connection with a two-way stop-cock *C*, by means of which the current of water can be shunted either to the heater, *H*, or in the case of a cooling experiment to a worm (not represented in the figure) placed in a freezing mixture and substituted for the heater. In this way a constant stream of any desired temperature may be obtained.

The heater consists of about 30 feet of small brass pipe wound into a coil and placed over a small Bunsen gas-stove. The stove and coil are entirely surrounded by the screen (*Scr*) provided with its chimney, *H*. Before the water passes to the heater, however, it is made to go through *Ww* to a worm of 25 ft. of ¼-inch bore lead pipe put in the hot water of the pan which forms the floor of the moist chamber. After traversing this worm it comes to the heater through *Hw*. In this manner the stream of water can be warmed even before it reaches the heater. The temperature of the water may be regulated by withdrawing the worm totally or in part from the water in the floor of the chamber, by the amount of gas burned, and by the rate of flow of the water. Of course in a cooling experiment this second worm was not used.

After issuing from the heater (or, in the case of cooling experiments, the worm in the freezing mixture) the stream is directed by *Rw* through the registering apparatus (*Rg*) where its temperature is ascertained. This apparatus is simply a T piece, one leg of which carries a thermometer. From this the water passes up *Bw* (Plate XXV) to the jacket *B* (Plate XXIV) and is then distributed over the whole system just described.

Dr (Fig. 1, Plate XXV) is a small sliding door which gives the assistant access to the interior of the case during the experiment. While an experiment is going on, the main doors of the moist chamber are opened as little as possible.

As will be seen, the cannula in Plate XXIV differs in detail from that shown in the diagram Plate XXIII. It is made in two pieces. One, *a*, is inserted in the aortic stump, and receives blood from the Mariotte flask. By its side branch *k* it was in some cases connected with the manometer of a kymograph. The other piece, *i*, is inserted in the innominate artery and contains a thermometer bulb. From it the tube *e* carries off the excess blood to the funnel *R*.

An attempt was made at first to get a graphic record of the pulse, by connecting a manometer with the aortic cannula; but the quantity of blood reaching the left ventricle was so small that the pulse waves due to its beat were indistinct and sometimes imperceptible. We had therefore to resort to counting by direct observation of the heart, which may have led to slight errors when the pulse was very fast and very feeble, but as our object was not to study the absolute pulse rate, small errors in regard to it were of no great importance. Moreover, by always taking two counts immediately following one another the chances of error were greatly lessened. First, one of us held the watch for thirty seconds while the other counted the heart beats. Then the duties were reversed and the other made the count, still being ignorant of the number

arrived at by the previous counter. If the two counts did not agree, but differed only by three or four in a minute, their average was put down in the tables as the correct rate; if the difference was greater (as was sometimes the case when rapid changes of temperature were occurring) a fresh count was made or the observation rejected altogether.

In all cases at least half an hour was allowed to elapse after complete isolation of the heart before the actual experiment commenced. This was in order to eliminate possible disturbing elements, as irritation of the vagi or accelerators, due to the operative procedure, or the first effects on the heart itself of the defibrinated blood. The general results of the experiments can be stated in few words.

First, as to cooling. The isolated heart of the cat may be cooled down to a temperature of 16.5° C. (as indicated by a thermometer introduced into the right heart) and yet not be killed, as it revives if soon warmed again (see Table I, p. 282), but it usually dies at about 17° or 18° C.

As the cooling proceeds, the pulse becomes slower and slower; this has two causes. As pointed out in previous papers, after about the first half-hour the pulse rate of the isolated heart tends to become slower the longer its isolation; so that a dog's heart isolated for one hour and beating, say, 220 times a minute at a temperature of 39° C., will at the end of two hours and at the same temperature beat only 200, or perhaps 190 times a minute. But the slowing in such cases is very gradual, and the heart will beat vigorously for four or five hours if not cooled. When the influence of cold is added, the fall in the pulse rate is far more rapid, and death of the isolated organ, if the cold be continued, occurs much sooner than it otherwise would.

TABLE I.

Cooling experiment with recovery, June 13, 1888.

Adult cat narcotized with paraldehyde during isolation of the heart. Isolation completed at 3.30 P. M.

Time P. M.	Temperature C. in Right Heart.	Pulse per 1′.	Remarks.
h. m.			
4.08	34.0	183	
4.11	33.7	175	
4.14	32.7	158	
4.17	30.9	138	
4.20	29.9	124	
4.23	28.5	111	
4.26	28.5	92	
4.29	26.7	80	
4.32	25.7	74	
4.35	24.7	66	
4.38	24.0	61	
4.41	23.5	54	
4.44	23.0	53	
4.47	22.3	50	
4.50	21.7	40	
4.52	20.5	?	
4.56	19.9	?	
5.00	19.5	12	
5.04	19.2	11	
5.06	18.9	9	
5.12	18.6	9	
5.15	18.0	9	
5.20	16.5	5	Heating now commenced.
5.40	21.0	14	
5.45	24.0	20	
5.50	27.3	78	Contractions becoming very feeble.
5.56	29.7	85	
6.06	31.7	90	

The results obtained on heating the heart to or near to its death point are more complex than those observed on cooling, but also more interesting.

In the first place we found that there was an *optimum* temperature at which the isolated cat's heart, like that of the frog, beat quickest, any rise beyond this slowing the beat. It by no means follows, of course, that this optimum is the temperature at which it would do most work. Averaging the results of thirteen separate experiments, this optimum is 41.3° C., ranging between 43.3 and 40.6.

If one steadily and slowly heats the heart to the highest temperature to which it can be raised without dying, this lethal

temperature lies between 44.5° and 45° C. in the great majority of cases; but sometimes by working with care, one can raise this *maximum* as well as the *optimum* temperature. If as soon as the heat is beginning to slow the pulse, or the heart shows other signs of weakening, the organ be cooled a little for a short time and then heated again, it can often be raised to a higher temperature without being weakened, and the *optimum* temperature also raised. Table II will serve as an example of a simple maximum and optimum experiment, while in Table III is given an example in which the optimum is raised by slightly cooling the heart from near the lethal temperature and then very slowly heating it again. This power of the heart to rapidly accommodate itself to an abnormally high temperature was so surprising to us that we made repeated observations on the matter. Since then we have been informed by friends engaged in the practice of medicine that something similar is frequently seen in persons suffering from remittent fevers. A rise of temperature associated with considerable quickening of the pulse may, later in the disease, be associated with a less marked quickening.

When the temperature of the heart rises to about 40° C., even though the optimum be not yet reached, small increments of temperature often have no effect on the rate of beat of the isolated heart, or the pulse may even become slower: this no doubt is due to that natural gradual slowing of the pulse of the isolated heart, always occurring as time goes on, and referred to above.

Another phenomenon often observed in heating experiments is what we may call a long latent period. An increase of temperature may not show any effect on the pulse rate for a minute or two, then it comes on, though the heart may in the meantime have been slightly cooled. For this reason (even before the optimum is reached) variations in the pulse curve tend to lag a little behind the temperature variations which led to them.

TABLE II.

Simple heating experiment to show optimum and maximum. June 7, 1888. Adult cat. Paraldehyde. Isolation of heart completed at 11.15 A. M.

Time.	Temperature C. in Right Heart.	Pulse per 1′.	Remarks.
h. m.			
11.51	34.1	168	
11.54	34.8	174	
11.57	35.2	186	
12.00	36.5	188	
12.03	36.9	186	
12.06	38.8	189	
12.09	38.8	190	
12.12	40.0	200	
12.15	40.3	201	
12.18	40.5	200	
12.21	40.5	200	
12.24	41.2	203	
12.27	41.2	202	
12.30	41.0	200	
12.33	41.8	206	
12.36	42.5	209	
12.39	43.2	210	Optimum or probably rather above it.
12.42	44.7	160	
12.44	45.7	?	Pauses alternating with periods of very rapid beats impossible to count.
12.47	46.0		Fibrillar contractions.

TABLE III.

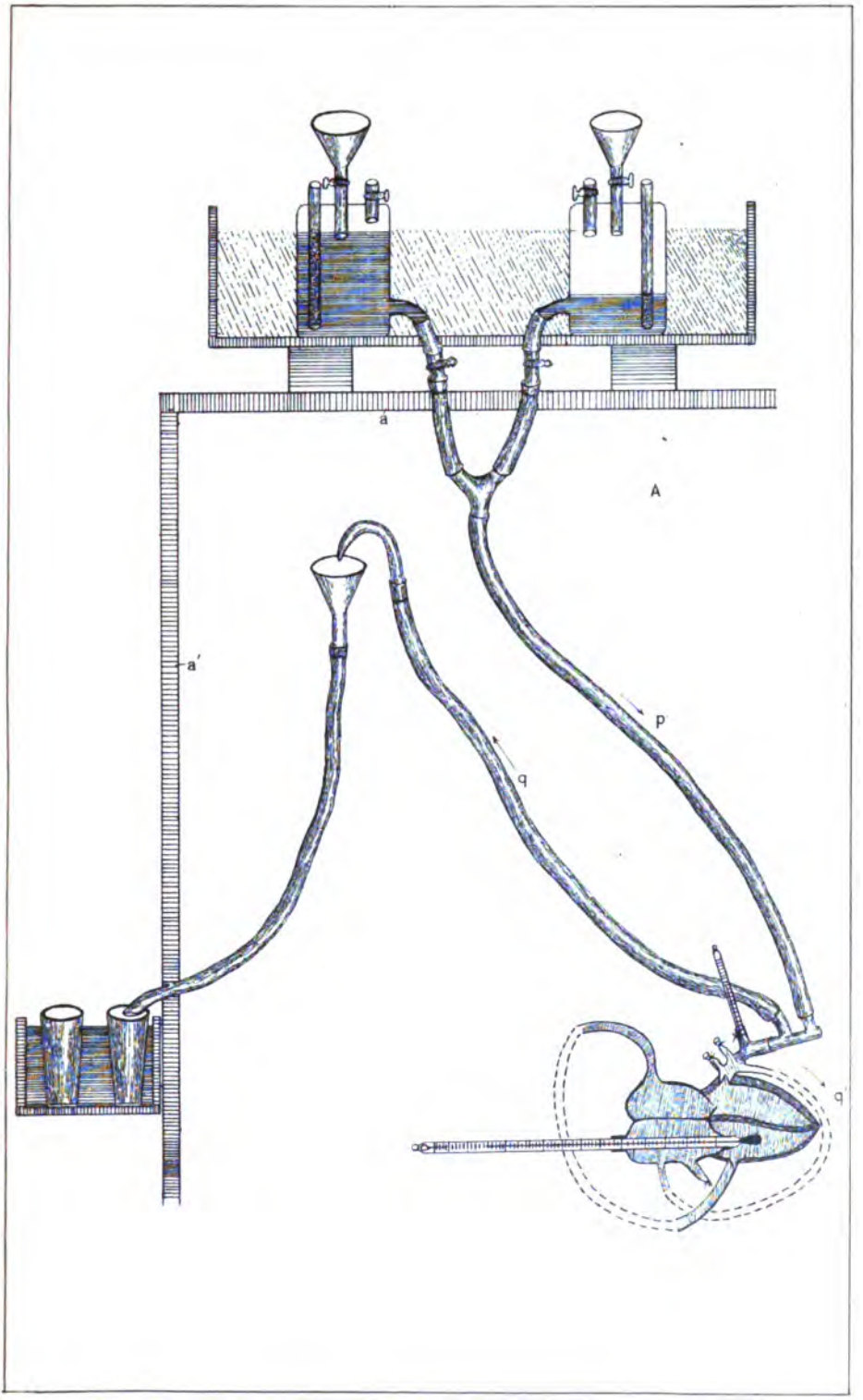
Optimum temperature raised during an experiment. January 31, 1889. Young cat. Ether and curare. Defibrinated blood used to feed the heart, diluted with one-third its volume of normal saline. Isolation completed at 2.55 P. M.

Time.	Temperature C. inside Right Heart.	Pulse per 1′.	Remarks.
h. m.			
3.51	39.3	234	
3.55	39.5	236	
3.58	39.5	220	
4.01	39.8	245	
4.03	40.3	240	
4.06	41.0	292	Temperature now raised with great
4.09	41.0	280	care, as heart is near its critical
4.12	41.0	272	point.
4.15	41.5	304	First optimum.
4.18	41.5	?	Irregular and not countable.

Time.	Temperature C. inside Right Heart.	Pulse per 1′	Remarks.
4.22	41.5	246	Regular. Heart apparently getting used to this temperature.
4.25	41.0	248	
4.28	41.0	204	
4.31	40.5	208	
4.34	41.0	204	
4.37	41.8	224	
4.40	42.0	?	
4.43	42.0	228	
4.46	42.5	234	Second optimum 1° C. above the first.
4.48	42.8	188	
4.51	43.3	198	
4.54	43.0	200 (?)	Somewhat irregular.
4.57	43.5	178	Regular.
5.00	43.0	178	
5.04	42.5	176	
5.07	42.8	176	
5.10	43.0	168	
5.13	44.5		Very irregular.
5.16	44.0	176	Regular.
5.19	48.0	172	Very irregular but not feeble beats; the irregularity probably due to the rapid change of temperature.
5.21			Only right auricle beating.

It is, of course, not possible to say at what temperature the heart of Table III would have died had the heat been slowly pushed on at $4^h\ 18^m$, instead of stopping and cooling from $4^h\ 25^m$ to $4^h\ 34^m$, thus giving the organ time to accommodate itself to the high temperature. But from many other observations in which the heating was slowly pushed on, we feel sure that all pulsation would have ceased at a temperature several degrees below 48° C.

It will be observed that though the temperature of the second optimum is higher than that of the first, the pulse is slower, this being no doubt due to the increasing malnutrition of the heart as time goes on.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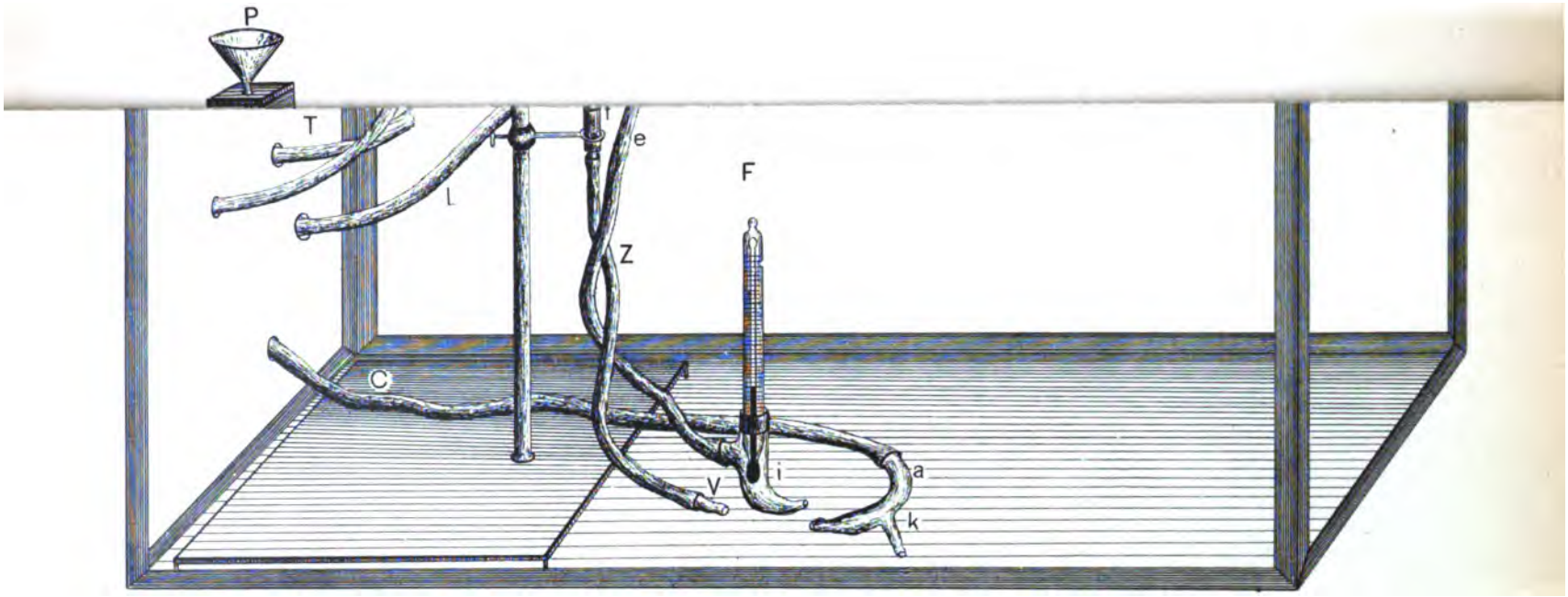
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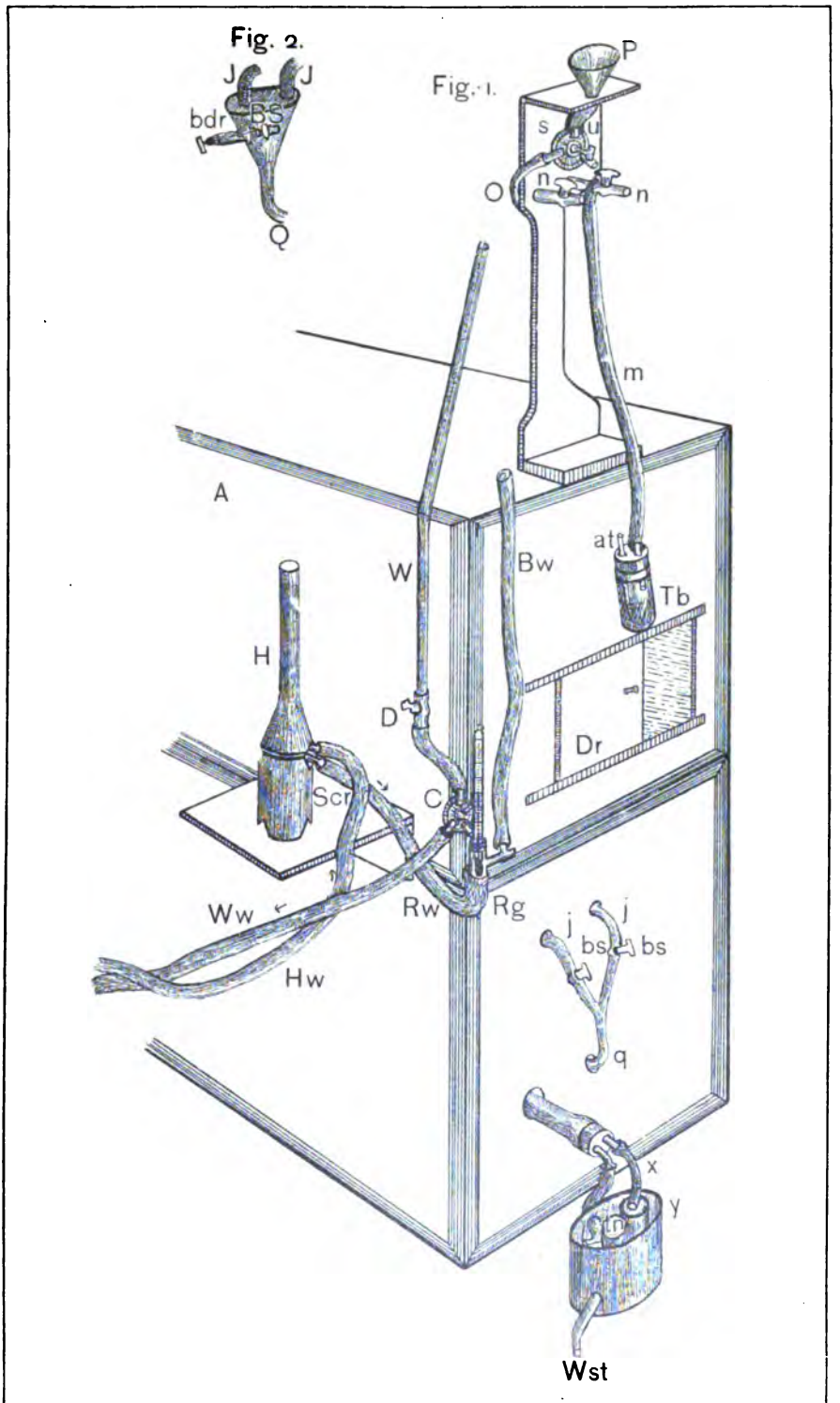
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ON THE MORPHOLOGY OF THE COMPOUND EYES OF ARTHROPODS.

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I.—INTRODUCTION.

Early in the spring of 1888, Prof. W. K. Brooks kindly placed at my disposal a valuable set of specimens of *Limulus*,[1] with a suggestion that I should work on the eyes in order to continue the research upon which the late Dr. A. T. Bruce was engaged at the time of his death.

Through the kindness of Prof. M. McDonald, the United States Commissioner of Fish and Fisheries, I was enabled to continue my work on the subject during the summer of the same year at the Woods Holl Laboratory of the Fish Commission, where the fresh specimens for the study of *Limulus* at all stages of development may be obtained without difficulty. To these gentlemen are due my sincere thanks and gratitude; through their kindness and encouragement I have been enabled to prosecute my

[1] These specimens were mostly preserved by Dr. J. C. Hemmeter, when the Chesapeake Zoological Laboratory was in session at Beaufort, N. C., in 1885. They were in an excellent state of preservation.

work. I am also indebted to Prof. John A. Ryder and Dr. E. A. Andrews for various courtesies.

It soon became evident that the eye of *Limulus* is, as we might expect from its great antiquity, a very primitive visual organ, presenting in a simple form a most interesting type.

For the further comprehension of its structure and significance it became necessary to extend my researches to several other Arthropods. Among the forms studied in this connection are the following, besides five other pelagic Arthropods from the Gulf Stream, whose generic names have not yet been ascertained: *Branchipus, Estheria, Talorchestia, Hyperia, Phronima, Serolis, Ligia, Asellus, Notonecta, Libellula, Agrion, Gryllus, Corethra, Cambarus, Homarus, Hippa, Alpheus, Gebia, Penaeus, Squilla, Callinectes, Gonodactylus* and *Lucifer*.

While only a few selected types are here described, all the above named forms, representing three great groups of Arthropods, Insects, Crustacea and Arachnids, were carefully studied, and all the results have been embodied in the general observations which follow, although the principal subject of the paper is the eye of *Limulus*.

The points which are at present receiving marked attention among the workers on the morphology of the compound eyes of Arthropods may be put in four principal categories, viz. (1) the mode of termination of the optic nerve fibre in the retinal cell; (2) the mode of formation of different "layers" in the retina; (3) the phylogeny of the compound eye; and (4) the homology of the optic ganglia.

The problem therefore involves several inquiries. The fundamental step is to determine to what morphological category the compound eye of the Arthropod belongs. To the elucidation of this point our histological inquiries must be directed, while our phylogenetic consideration of the eye may fitly be reserved until we have learned something of its structure.

The first thing which confronts us is this: What is the ommatidium or "eyelet," the repetition of which, often several thousandfold, gives rise to the compound eye? What is the significance of the characteristic arrangement of its component cells? Is it possible to reduce it to a simpler form, or to express its structure and significance briefly?

Until these questions be answered satisfactorily, the most laborious technique of histology is often powerless to discriminate the essential from the secondary features in the component cells of a given ommatidium; and in the absence of an accurate conception of its fundamental structure, the various modifications of the cells and fibres of an ommatidium may be made to assume any significance to suit our speculative ideas.

With these considerations in mind, I have sought for an ommatidium in which all the features of its structural elements may be made out plainly, and from which we may derive the ommatidial elements of a more complex type.

Although the number of forms studied in this connection is not quite so extensive as I wish, I feel justified in believing that any view which explains the structural peculiarities of the ommatidia of each and all of the forms in the list above given and reduces them to the same fundamental structure, may fairly be considered as in all probability generally applicable to the eyes of Arthropods.

In what follows, the attempt will be made to approach the subject from this standpoint, viz. the consideration of the ommatidium as the morphological unit of the compound eye in Arthropods, just as each little circle of rods with a cone in its centre may be considered as the morphological unit of the "mosaic layer" (Henle) of the human retina.

II.—The Morphology of the Ommatidium.

Following the plan given in the Introduction, I will consider the morphology of the ommatidium, taking it as the structural unit of the compound eye. After the nature of this unit has been considered, we may proceed to examine the state of aggregation of the units in the different forms of the Arthropods. For this purpose it is convenient to select one particular ommatidium which may be made the basis of comparison. Accordingly, the ommatidium of *Serolis* will be considered at the outset, and the attempt will be made to homologize the ommatidial elements of *Serolis* with those of other forms. I am indebted to Prof. M. McDonald for the opportunity to study the eyes of the four South American forms of *Serolis* recently brought back by the U. S. Fish Commission steamer *Albatross*.

a. Serolis.

(Figs. 1, 1*a*, 1*b*, 2, Pl. XXIX.)

The general structure of the eyes of *Serolis* has already been made known by Beddard.[1] Although I have verified all the chief results of his research, I shall speak of a few details which seem to me specially significant.

The cells of the ommatidium are arranged in three principal strata, *a*, *b*, *c* (Fig. 1*a*, Pl. XXIX). Each stratum of cells is characterized by the special product which it secretes, viz. 1, 2 and 3.

The outermost stratum (*a*) secretes upon its external surface the chitin which constitutes the *cornea* or the *corneal facet* (*c*, Fig. 1, Pl. XXIX).

The cells themselves have been designated as the *corneagen* by Patten.[2]

The corneagen is sometimes difficult to detect in the eye of the adult animal and may easily be overlooked. It is very conspicuous in the young.

The next stratum of cells (*b*) is also characterized by the chitinous secretion on the surface nearest the median axis of the ommatidium. The two cells (*b*) secrete a thick chitinous layer which encloses an ovoidal space of considerable size filled with a transparent liquid substance, (2). The cell (*b*) has been named *vitrella* (Lankester and Bourne);[3] and the ovoidal chitinous body (2) is the *crystalline cone*[4] (*c. c*, Fig. 1, Pl. XXIX.)

[1] Beddard, F. E. Challenger Report, Zoology, Vol. XI, *Isopoda: Serolidae.* His additional observations on *Serolis* may be found in the paper by the same author, *On the Minute Structure of the Eye in Cymothoidae*, Trans. Roy. Soc. Edin. 1887.

[2] *Studies on the Eyes of Arthropod:* I. *Development of the Eyes of Vespa, with observations on the Ocelli of some Insects*, p. 193, Journal of Morphology, Vol. I, No. 1, 1887.

[3] *The Minute Structure of the Lateral and the Central Eyes of Scorpio and Limulus.* Quart. Jour. of Micro. Science, Vol. XXIII, p. 198, 1883.

"Vitrella" is synonymous with "retinophora" of Patten; "vitreous cell" and "crystalline cone cell" of authors. The nucleus of the vitrella has been named by Claparède "Semper's nucleus," after its discoverer: *Zur Morphologie der zusammengesetzten Augen bei den Arthropoden.* Von Edouard Claparède. Zeit. f. Wiss. Zool. Bd. X, p. 193, 1860.

[4] It is interesting to notice that the observations made by Clarke on the eye of a Trilobite (*The Structure and Development of the Visual Area in the Trilobite,*

The crystalline cone is purely a dioptric apparatus. A careful study of its structure, both in the adult and in the young embryos of *Serolis*, has clearly shown that this has nothing to do with the sensory function, as it has no connection whatever with the optic nerve fibres.

The vitrella, then, agrees with the corneagen cell in the capacity for secreting chitin on one portion of its surface. The difference between them consists in this, that while the corneagen cells secrete chitin towards the exterior of the body, the vitrellae secrete it towards each other, that is, towards the median axis of the ommatidium.

Next below the vitrella comes the important stratum (*c*, Fig. 1*a*) which lies at the bottom of the whole structure, and which sends processes inward (*Op. n*) to form the optic nerve fibre, which terminates in the optic ganglion.

The cells in this structure are also characterized by secreting chitin towards the median axis of the ommatidium. The chitin thus produced invests the colorless portion of the cell (*c*) constituting the rod or the *rhabdomere* (Lankester and Bourne).[1]

A large number of transverse striae, running at right angles to the longitudinal axis of the ommatidium, are seen through the transparent cuticle. The cell itself which gives rise to the rhabdomere has been known as *retinula* (Grenacher).[2] The

Phacops Rana, Green, Journal of Morphology, Vol. II, No. 2, 1888) show, so far as I understand his account, that the lens has a somewhat similar structure to the crystalline cone of *Serolis*, or, in fact, to the crystalline cone of Isopod crustacea, as far as it is known. From Clarke's description I gather *that the lens of Phacops is unequally biconvex, the curvature being the greatest on the proximal surface; and that the lens was hollow, probably filled with some viscid humor.* In the absence of a more complete knowledge on the nature of a corneal covering to the eye in the Trilobite it is premature to carry out the homology of the "crystalline cone" of *Serolis* and of the Isopods in general to the "lens" of a Trilobite, although there appears to exist a certain resemblance between the two. It is perhaps worthy of mention, in this connection, that there is nothing whatever in the lens-cone of *Limulus* at any period of its development which shows any resemblance to the lens of a Trilobite as made out by Clarke. *The lens-cone of Limulus is the solid, conical projection of the chitinous cuticle of the body, fitting into the open depression of the skin, and in no period of the life history of the animal is it separated from the outermost cuticle of the body.* (Compare Grenacher, Lankester and Bourne, and Part III of the present paper on the compound eye of *Limulus*.)

[1] *loc. cit.* p. 183.

[2] *Untersuchungen über das Sehorgan der Arthropoden*, 1879.

retinula, then, agrees with the corneagen and vitrella w
above, in its capacity to secrete chitin on a part of its
Thus the three strata of cells, *a*, *b*, and *c*, agree fundan
with one another in their capacities to secrete chitinous str
and these are respectively known as the *cornea* (1), the
line cone (2), and the *rhabdomere* (3).

Hence, aside from the general homology which exists
the cells in the optic area, all being derived from a
source, the ectoderm, there exists a special homology
the cells in the three strata which constitute the importa
of the ommatidium, all being characterized by the capa
secreting chitin from a part of their surface.

In this respect these ommatidial cells are essentially
general ectodermal cells which secrete the chitinous cov
the body from their distal extremities.

In carrying this analysis further we find that the outer
of the corneagen is homologous with the axial surfac
vitrella, and that of the retinula and the three chitinous str
cornea, *crystalline cone* and *rhabdomere*, are homologo
each other.

If this interpretation be established by the further di
of the ommatidia of other forms, it follows that the cr
cone and rhabdomere are really homologous with the c
investment of the body, and the narrow axial space
ommatidium represented by the line *x* must therefore
same significance as the space outside of the body. M
gically speaking, the crystalline cone and rhabdomere
as much a part of external surface as the cornea itself. I
atically, the three strata of the ommatidium may be rep
as in Fig. 2, Pl. XXIX.

According to this view, the ommatidium of *Serolis*
regarded as an open pit of the ectoderm, the walls and
of which are covered by the product of their own secre
chitin. The chitin in the walls and margin of the pit
continuous with the general chitinous covering of the
the rows of the ommatidial cells themselves become co
with the general ectoderm.

At the bottom of the ommatidial pit there exists a
colorless "hyaline cells" (Beddard), with their upper p

inserted into the axial space between the four rhabdomeres. Each cell has a peculiar refractive nucleus. It lacks any connection with the optic nerve fibre, and is suspended from the bottom of the pit at a considerable distance above the basement membrane. The "hyaline cell" is a modified ectodermic cell, and is homologous with the rest of the cells which enter into the formation of the ommatidium. The cell, however, does not develop pigment granules in itself, nor establish any connection with the central nervous system as does the retinula cell, nor does it secrete chitin on a part of its surface as do the rest of the ommatidial cells we have considered already. As to the function of this cell I am not able to say anything definitely. It cannot be sensory, having no connection with the nerve centre. When we see, however, that in some species of *Serolis* the lower ends of the four rhabdomeres are deeply imbedded in the substance of the "hyaline cell," or, in other words, that the rhabdomeres are firmly held together by the substance of the "hyaline cell" at the point where they meet, it becomes probable that the cell in question may serve the purpose of a mechanical support, adding firmness to the whole structure. To this point I will recur later on.

In addition to the cells which form the essential part of the ommatidium there exists a number of pigmented cells, (*pg. c.* Fig. 1*a*, Pl. XXIX). The pigment cells are greatly developed around the vitrellae and closely invest the dioptric portion of the ommatidium from outside. These pigmented cells also are modified ectoderm cells which lie between the adjacent ommatidial pits.

The morphological unit of the compound eye of *Serolis* may then be described as consisting of a group of ectodermic cells lying outside of the basement membrane, and so arranged as to form the walls of an open tubular depression. This group of cells undergoes special differentiations at different levels of the pit. The uppermost group, consisting of two flattened cells, constitutes the corneagen, which secretes the cornea externally; the second group of cells, consisting of two large cells, the vitrellae, secretes chitin towards the lumen of the tube, thus forming the crystalline cone; the third group of cells, the retinulae, consisting of four cells, secretes the rhabdomeres. The retinulae

are the only cells which are connected with the nerve centre. In addition to the three elements above named, the fourth element, in the form of two "hyaline cells," exists, plugging, as it were, from the inside the imperfect bottom of the depression. A number of pigment cells enveloping more or less completely the above mentioned group of cells from the outside constitutes the fifth element of the ommatidium.

A glance at the diagram (Fig. 1*b*, Pl. XXIX) will make this point clear. The diagram is supposed to be the plan of the constructive elements of the ommatidium of *Serolis* as they would appear when seen from the exterior, in the direction of the arrow, Fig. 1*a*, Pl. XXIX, if they actually formed an open pit like that shown in Fig. 2.

The innermost body which appears in the depression is the pair of "hyaline cells," (*d*); next above and therefore in the outer circle to (*d*) are the retinulae (*c*), the rhabdomeres being represented by the yellow edges. Above the retinulae will come the two extremely enlarged cells, the vitrellae (*b*), whose chitin-secreting surface is marked by the yellow color. Next above (*b*) comes a pair of corneagen cells (*a*), whose chitin-secreting surface is also represented by the yellow color as in the cells forming the two inner series. The outermost of all is a pair of extremely flattened, pigmented cells, ensheathing completely the dioptric portion of the ommatidium.

The diagram is not unlike the one used in illustrating the arrangement of the different parts of a flower. This does not seem surprising when we remember that the nature of the problem in both cases is identical. Just as sepals, petals, stamens and pistils of a flower are considered as the modified leaves clustered round an extremely short stem or axis in whorls or in spirals, so the corneagen, vitrellae, retinulae, etc., are the modified ectoderm cells arranged at different heights on the ommatidial axis. There is, however, this difference between them, that while in a flower the parts concerned in the formation of the organ are themselves aggregations of many cells, those in the Arthropod ommatidium are highly differentiated individual cells; and while in the case of the flower it is the clustering of different parts around an extremely shortened projecting axis, in the ommatidium of the Arthropod it is the distribution of parts along an elongated and sunken one.

Although the subject will be much more intelligible after other types have been described, it will not be altogether out of place here to mention briefly my view of the steps through which the characteristic arrangement of parts in an ommatidium like that of *Serolis* might have been brought about. Suppose a circular area of the skin to be divided into five zones, one lying within the other. Mark the innermost circle as (1), and the next zone as (2), and the middle as (3), the fourth as (4) and the outermost as (5). Suppose, further, this circular area of the flat ectodermal surface of the body to sink down as a conical pit with its open base turned towards the exterior. The cell or cells lying in the innermost circle (1) in the middle of the area will sink deepest, while the outermost circle (5) will retain its original level. The cells lying in the innermost circle (1) are the "hyaline cells"; those lying within the second zone (2) are the retinulae; those lying in (3) are the vitrellae; those within (4) are the corneagen, and those within (5) are the group of pigmented cells which surround the dioptric portion of the ommatidium.

All that is necessary to convert this diagram into the ommatidium of *Serolis* is to reduce the lumen of the pit until it finally disappears and the cells facing the lumen of the tube come into contact with each other, or else remain separated by the chitinous structure; a faint line like that shown by x in Figs. 1, 3 and 4 being all that remains of the axial cavity, like that shown in Fig. 2.

The compound eye of *Serolis* is in this view nothing but a collection of these depressions in the skin, which by virtue of their aggregation attain the morphological and physiological value of an organ.

b. Talorchestia.

(Figs. 3, 3*a*, Pl. XXIX.)

A glance at the figures will at once show the fundamental similarity of the ommatidium of this Amphipod and that of *Serolis*. Fig. 3, Pl. XXIX, shows a single ommatidium of *Talorchestia*. The outermost covering c is the corneal facet. The two cells which lie beneath it are the corneagen ($c.g$). These cells are not so conspicuous in the adult as in the young

animals; nevertheless, with care one can easily make them out. Beneath the corneagen comes the stratum consisting of two cells, the vitrellae, which secrete an enormous cuneiform, chitinous structure, the crystalline cone (*c. c*) with its more pointed end turned inward. The crystalline cone consists of halves, each corresponding to a single vitrella cell which secretes it on its median, axial surface. The line *x*, as in *Serolis*, shows the morphological lumen of the ommatidial cavity.

Next below this stratum comes the group of four cells, the retinulae (*Rt*). The chitinous edges of the retinulae meet in the median axis and form the rhabdom (*Rb*). Transverse striations, rather broad and coarse in their nature, are seen through the transparent cuticle, the rhabdomere. At the lower part of a retinula cell, near the basement membrane, is situated its nucleus (*n*); it may be found below the membrane.

The retinula cell undergoes a curious modification at the upper end. Each of the retinula cells sends out a flattened process which is highly pigmented and which completely ensheaths the dioptric portion of the ommatidium from the outside. In fact, the place of pigment cells (*pg. c*) in *Serolis* is taken by the modified upper expansion of the retinula cells. Outside of this pigmented sheath there exists a number of non-pigmented, elongated ectodermal cells (*p. c*) which pack the interspaces between the adjacent ommatidia.

If we plot out the whole structure into a diagram, in the same way as we did the ommatidia of *Serolis*, it will assume the appearance shown in Fig. 3*a*, Pl. XXIX. The innermost cells, the "hyaline cells" of *Serolis*, are not represented; the cells in the second circle are greatly developed (*Rt*), meeting one another with their chitin-secreting edges, the rhabdomeres, while the upper extremity of each retinula cell envelops the crystalline cone from the outside, thus physiologically taking the place of pigment cells, (*pg. c*) or 5 in *Serolis*, Fig. 1*b*, Pl. XXIX.

In the third circle the two vitrellae are found with their chitin-secreting surface marked yellow. The cells in the fourth circle constitute the corneagen (*c. g*). The fifth circle of cells is represented by the packing cells (*p. c*).

c. Cambarus.

(Fig. 4, Pl. XXIX; Fig. 35, Pl. XXXI; Figs. 71, 79, Pl. XXXV.)

In Fig. 4, Pl. XXIX, three ommatidia of *Cambarus* are represented, *a*, *b* and *c*. The ommatidium may be described as in the two preceding cases, as consisting of three strata of cells, each stratum characterized by the special chitinous product which it secretes. The outermost stratum of cells, the corneagen (*c. g*)[1] secretes a slightly biconvex corneal facet (*c*).

Next below the corneagen comes the stratum of vitrellae, consisting of four cells, each with an extremely elongated internal process. The median surface of the vitrella which corresponds to the chiting-secreting surface of the vitrella in *Serolis* and *Talorchestia* secretes a perfectly transparent, homogeneous substance, rather refractive in its nature, forming the crystalline cone (*c. c*). I cannot say anything of the chemical nature of this substance in its relation to the chitin. The cells which secrete this substance in the crayfish are morphologically identical with the vitrellae in *Serolis* and *Talorchestia* which secrete chitin, and the use this body subserves in the ommatidium of the crayfish is identical with that of the chitinous body, the crystalline cone, in the two Arthropods we have already examined. There can be no question of the homology of this body to the crystalline cone of other Arthropods. I have therefore represented this body in the yellow color as in *Serolis* and *Talorchestia*. The outline of the vitrella cell in the figure has been made diagramatic to a certain extent.

Next below the vitrellae come the retinulae, the whole being aggregated into a spindle-shaped bundle.[2] On the distal end of

[1] Reichenbach (*Studien zur Entwicklungsgeschichte des Flusskrebses*, p. 93) speaks of these cells as "Semper'schen Zellen." In examining his figure (Fig. 225, Taf. XIV) we find what he regards as "Semper's cells" are those with "Semper'schen Kerne" (S. K.) The term "Semper's nucleus" as originally used by Claparède and adopted by others does not mean the nucleus of the corneagen, as Reichenbach uses it, but means that of the vitrella. In fact the existence of corneagen has been ignored by several earlier writers. It was Patten who emphasized its importance.

[2] This spindle stratum does not come from any part of the "augenfalte" of Reichenbach, but arises in the region where he locates the ectodermal and mesodermal pigment cells, *loc. cit.* Fig. 225, Taf. XIV.

the rhabdom the proximal ends of the vitrellae apparently terminate.[1]

When the spindle is macerated and each of its component elements is examined individually, it is found that each retinula develops on its median surface a thick chitinous border which shows a wavy, corrugated appearance. This chitinous border attains its greatest thickness in the midst of the retinula cell (Fig. 35, Pl. XXXI). When the chitin-secreting edges of a number of these retinula cells meet they form a spindle-shaped bundle, the thickest part of the rhabdomere corresponding to the most bulging part of the spindle. The lower extremities of the retinula cells send out nerve processes, each of which forms an optic nerve fibre, (*Op. n*, Fig. 4, Pl. XXXI). Fine elongated cells filled with yellow pigment granules are found between the adjacent retinula bundles (*ep*, Fig. 4). They are also the modified ectodermal cells. The pigment cells which occur around the vitrellae form a complete envelope around the dioptric portion of each ommatidium.

Thus even with this complicated type of ommatidium the plan of structure is fundamentally alike to that of *Serolis*. The central element, the "hyaline cells" of *Serolis*, is not represented in the crayfish. To this special point I shall recur.

If we plot out the ommatidial elements of the crayfish as we did those of *Serolis*, exactly a similar kind of diagram will be formed. Thus in the centre will come the group of retinulae,

[1] Parker found in *Homarus* (*A Preliminary Account of the Development and Histology of the Eyes in the Lobster*. Proceedings of the American Academy, Vol. XXIV, pp. 24–25, 1888) that the lower extremities of vitrellae run over the spindle, and that each terminates on the retinal side of the basement membrane. It is probable that the same is true in *Cambarus*. This fact is perfectly intelligible according to the view which is maintained in the present paper, for the cells found in the higher level of a given ommatidium are morphologically situated in the outside of those found in the lower. Patten's account (*Eyes of Molluscs and Arthropods*, Mittheilungen aus der Zoologischen Station zu Neapel, Bd. VI, Heft 4, 1886) that the rhabdom is the continuation of the retinophorae (vitrellae) is not true. I macerated the "spindle" of *Penaeus*, upon which Patten bases his observations, and found that it is composed of a number of pigmented retinula cells, each with its chitinous border as in *Cambarus* (Fig. 35, Pl. XXXI) or in *Homarus* (Fig. 34, Pl. XXXI). Grenacher is right in regarding the rhabdom as the secretion product of the retinula cells.

consisting of seven cells; then come in the outside four vitrellae arranged in a square; then two corneagen cells; and outermost of all, four pigmented cells which completely envelop the dioptric part of the ommatidium.

d. Homarus.

(Fig. 34, Pl. XXXI.)

The compound eye of *Homarus* has quite recently been studied by Parker.[1] I do not find any facts in Parker's paper which contradict the view maintained in the preceding pages in regard to the nature of the ommatidium. The threefold differentiation of the ectodermal cells in the walls of the ommatidium is just as evident here as in the forms already considered. The problematical body, the spindle (Grenacher's rhabdom), like the corresponding structure in *Cambarus*, resolves itself into a number of individual retinula cells with thick, serrated, chitinous secretions as in the case of *Cambarus* and *Penaeus*.

In Fig. 34, Pl. XXXI, four retinula cells are partly isolated from one another. The lower end of each retinula cell is prolonged into an optic nerve fibre, while the upper end is devoid of any pigment granules. A large translucent nucleus exists near the distal extremity of the retinula. The distal end of each cell is prolonged into a short, clearly defined, tapering process. The development of the chitinous serrature is greatest in the middle of the chitin-secreting border of the retinula; when, therefore, the whole is brought together into a bundle, the general outline of such a structure is spindle-shaped.

e. Callinectes.

(Figs. 5, 5*a*, Pl. XXIX; Fig. 37, Pl. XXXI; Fig. 72, Pl. XXXV.)

Callinectes presents the same interesting modification which we saw in *Talorchestia*. We find the corneagen cells and the vitrellae in two successive strata, but no special pigment cells embracing this dioptric mechanism exist. The packing cells which we saw in the intervening spaces of the ommatidia in *Talorchestia* are not found in *Callinectes*.

[1] G. H. Parker: *A Preliminary Account of the Development and Histology of the Eyes in the Lobster.* Proc. Amer. Acad. Vol. XXIV, 1888.

The third stratum of cells, the retinulae, are extremely elongated, each retinula with a clear, straight, finely striated rhabdomere. A somewhat diagramatic representation of the individual retinula cell with its chitinous border is given in Fig. 37, Pl. XXXI. In Fig. 5*a*, Pl. XXIX, the upper portions of the two retinula cells are shown; the rhabdomere just beneath the crystalline cone shows a slight enlargement. The size of the retinula cell at the distal extremity undergoes an enlargement. The enlarged extremities of the retinulae form a pigmented collar around the inner end of the crystalline cone. If the upward extension of this part of the retinula cells be carried still further, a condition which prevails in a retinula like that of *Talorchestia* will be found. In the freshly teased preparation, one or more refractive globules, ranging from yellow to reddish brown in color, are found in the thickened portion of the retinula. The general shape of the ommatidium as determined by the shape of the corneal facet is hexagonal.

For the general discussion of the subject I do not find it necessary to go into further details of comparison, nor to enumerate more examples from different Arthropods. In the forms I have studied in this connection, representing near thirty Arthropod genera, I found nothing which invalidated the general interpretation of the ommatidial structure of the Arthropod as explained in the preceding pages, and the compound eye of the Arthropod, whether it be sessile or stalked, must be held to represent morphologically a group of ectodermal pits or a bundle of ectodermal tubules.

III.—The Compound (Lateral) Eye of Limulus.

The question which next suggests itself is this: Adopting for a moment the view that the ommatidium of the compound eye is morphologically an ectodermic pit, is there any adult arthropod whose eye permanently remains in this condition?

I find such an ommatidium in the compound eye of *Limulus*.

On this account I will describe the eyes of *Limulus* somewhat in detail.[1] In the succeeding section of the paper I will point

[1] An outline of this portion of the paper will be found in the March number of the *University Circular*, 1889, Johns Hopkins University, under the title *The Structure and Development of the Eyes of the Limulus.*

out how an ommatidium like that of *Limulus* may be converted into one of a more complex type.

a. General Sketch of the Compound Eye.

The compound eye of *Limulus* is placed in the dorso-lateral angle of the prosomatic shield. In the fully grown animal the outline of the eye is bean-shaped, with its longer axis parallel to the longitudinal axis of the body. This bean-shaped area is slightly protuberant from the surrounding level; the chitinous covering of the body is thinner, softer, and more translucent in the region of the eye than on the rest of the dorsal surface. Through the translucent chitin we see a large number of circular spots, all of a uniform size. On further examination we find that each spot corresponds to a conical projection of the chitin, which fits into a cavity in the skin. This conical thickening of the chitin constitutes the *lens* or *lens-cone*, and the group of cells forming the walls and bottom of the open cavity constitute the essential part of the ommatidium.

The cells which form the walls of the pit are elongated and highly pigmented, forming the perineural cells (*p. n*, Fig. 6, Pl. XXIX); those cells which form the bottom of the pit undergo extreme enlargement compared with the perineural cells, and elongate along the longitudinal axis of the pit. They are grouped in a characteristic manner, reminding one of the arrangement of cells in the taste-bulb of a vertebrate. This bulb-like group of cells is the essential part of the retina. In my preliminary account of the subject[1] I called this group of cells an ommatidium. Properly speaking, the term ommatidium includes the whole group of cells forming the walls and bottom of the pit, together with the lens-cone and the thick chitinous covering corresponding to the area occupied by a single pit. In this latter sense the term ommatidium will be used in the present paper, and the bulb-like group of cells at the bottom of the pit will be designated as the *sensory part of the ommatidium.*

In the sensory part of the ommatidium, which consists of two portions, a central *a* and a peripheral *b*, the arrangement of the cells is quite regular.

[1] *loc. cit.* p. 70.

The central part always consists of a single ganglion cell (*G*, Fig. 6, Pl. XXIX), and the peripheral of the rod-bearing pigmented cells (*Rt*, Fig. 6) which surround the former in the centre. As to the minute structure of these elements I shall speak later on. The cells composing the sensory portion of the ommatidium send out from their proximal ends nervous processes, which, forming a bundle, are seen to emanate from the basal end of the ommatidium. The number of nerve fibres at the beginning of the bundle and that of the cells composing the sensory bulb of the ommatidium always correspond. These bundles, however, soon break up as they penetrate deeper, and become mixed up with the fibres from the neighboring bundles, forming a complex plexus underneath the ommatidial area. Scattered in the plexus are found a number of thickenings, which, in gold-chloride preparations, appear as darkly stained masses of protoplasm occurring at the nodes of junction of the several intercrossing fibres (Figs. 44 and 45, Pl. XXXII). In the fresh state these enlargements contain a variable amount of yellow granules imbedded in the mass of the protoplasm. In the figures above referred to, mere outlines of such thickenings are given.

After the nerve fibres have formed the plexus they again come out in bundles, and piercing through the perforations in the basket-like chitinous support which underlies the ommatidial region, travel forward, slightly inward, then downward and backward until they terminate in the optic ganglion in the brain.

b. The Structure of the Ommatidium.

In the general sketch of the compound eye of *Limulus* above given, the structure of its morphological unit, the ommatidium, has been briefly described. It consists of two parts, (1) the sensory and (2) the dioptric part, which is also partly protective.

The sensory part consists of two factors, (*a*) the central element or the ganglion cell (*G*, Fig. 6, Pl. XXIX), and (*b*) the peripheral elements or the retinulae (*Rt*, Fig. 6). These two kinds of cells are the only ones in which nerve fibres terminate.

The retinula cells group themselves in the shape of a bulb, or something as the several segments do in an orange, each segment corresponding to a single rod-bearing cell or retinula (Fig. 10,

Rt, Pl. XXX). Along the axial side of the retinula cell there exists a definite longitudinal tract which is free from the pigment granules. On the outside of this colorless region there exists a thin layer of chitin. This chitinous covering on the outside of the retinula constitutes the rod or the rhabdomere (*Rb*, Fig. 10, Pl. XXX). When the non-pigmented portion of the retinula cell is examined with a high power, we find the whole structure to be transversely striated. This striated appearance seems to be produced by the existence of an enormous number of transverse fibrils lying in the inside of the chitinous cuticle. That these striae are due to the existence of independent fibrils is further shown by the fact that by careful focusing up and down of the microscope some striae can be directly traced out beyond the limit of the chitinous covering, in the form of independent fibrils (*Tr. fb*, Fig. 10, Pl. XXX).

When we make a transverse section of the retinulae, each cell will present a wedge-shaped outline, with its pointed end turned toward the axis of the ommatidium; the non-pigmented part of the cell in its relation to the cuticular secretion on the outside will be seen from the diagram (Fig. 6*a*, *b*, *Rb*, Pl. XXIX).

The number of retinula cells entering into a single ommatidium is quite variable. Thus, Grenacher[1] counts fifteen; Lankester and Bourne[2] count ten; I have found some with eleven, others with nine; in one ommatidium I counted as many as nineteen.

I am not able to say under what conditions this numerical variation takes place. Whatever significance there may exist in this numerical variation, it does not alter in the least, as in the case of different elements in other ommatidia we have already considered, the general idea that the ommatidium of the Arthropod is morphologically a depression in the skin.

Each ommatidium contains a single ganglion cell. Only in one case did I see two ganglion cells in a single ommatidium. The ganglion cell is situated morphologically in the centre of the sensory part of the ommatidium. The retinula cells completely envelop this ganglion cell from the outside, in a similar way as the rods encircle the cone in the centre in the retinae of some vertebrates.

[1] *loc. cit.* Fig. 125, Taf. XI.

[2] *loc. cit.* Fig. 10, Pl. XI.

The main body of the ganglion cell where the nucleus is situated is found in the lower part of the ommatidium, occupying in most cases an eccentric position. Figs. 13, 14 and 15, Pl. XXX, show three ganglion cells completely isolated from the rest of the retinal cells. In Fig. 10, Pl. XXX, the relation of the ganglion cell to the retinula cell is well shown.

The ganglion cell is distinguished by constant and well marked features from the rest of the ommatidial cells. The cell is bi-polar, the one process going upward and outward through the axial channel of the ommatidium (the axial canal of the rhabdom), and the other going downward and inward to the sub-ommatidial plexus and thence to the brain. For the sake of convenience the former will be designated as the axial process (*Ax. p*, Figs. 13, 14, etc.), and the latter as the optic nerve process (*Op. n*, Figs. 13, 14, etc.) of the ganglion cell.

The axial process gradually tapers to a narrow point. The process consists of a bundle of extremely fine fibrils, which are distinctly seen as a series of longitudinal striae. For some mechanical causes, the distal process was seen split into three main branches in one specimen (Fig. 15, Pl. XXX); in another specimen (Fig. 10), the topmost end of the axial process was seen completely macerated into a number of component fibrils, the whole showing a brush-like appearance.

The large, translucent, spherical nucleus (*N*, Figs. 10, 13, 14, 15, Pl. XXX), with its nucleolus, is found in the substance of the cell body proper. The position of the nucleus is very often eccentric, as in Fig. 14. The nucleolus is surrounded by concentric markings in all cases. Between the nucleus and the proximal process there is always a larger or smaller patch of pigment granules (*Pg. p*, Figs. 10, 13, etc.). The color of the granules ranges from light yellow to dark brown or even to jet black. The yellow-colored pigment is diffused more or less widely through the body proper of the ganglion cell. Even in the same patch of pigment in the cell there are different gradations in the color. From the nature of the drawing used in the present paper I cannot show these gradations. Pigment patches may exist in other parts of the cell, but they never form so constant a characteristic as the group between the proximal process and the nucleus.

The third element which figures prominently around the sensory cells of the ommatidium is the eipithelial cell. The cell may either be pigmented or non-pigmented. In Fig. 11, Pl. XXX, a group of four retinula cells is shown partly isolated from one another. Within the interspaces between the adjacent retinula cells will be found a number of pigmented epithelial cells, extremely elongated, many of them extending the whole length of the retinula cell. In Fig. 12, Pl. XXX, a number of highly attenuated epithelial cells (*Ep*) is shown closely clinging to the outer surface of the retinula cell. In Figs. 10, 13, 14 and 17 highly attenuated epithelial cells (*Ep*), both of the pigmented and the non-pigmented kind, are shown, as they occur either around the ganglion cell or around the retinula cell.

In no case are these epithelial cells found to establish any organic connection with any part of the sensory cells or of their processes. These attenuated epithelial cells do not differ in any essential way from the ordinary ectodermal cells which are found in the walls of the ommatidium or in the general ectoderm.

The epithelial cells as they further depart from the periphery of the group of sensory cells, become stouter and thicker. One end of each epithelial cell is usually devoid of pigment granules; this non-pigmented end of the cell secretes the chitin which forms the lens-cone. We have already seen that the portion which secretes the chitinous covering, the rhabdomere, of the retinula cell is devoid of pigment granules. In this respect the epithelial cells which form the wall of the pit and the neuro-epithelial cells which form the bottom of the pit closely agree with each other.

A glance at the series of figures (Figs. 17–33, Pl. XXXI) will show the degrees of modification undergone by the epithelial cells teased out from the different parts of the ommatidium. Those cells (Figs. 17 and 18) which lie at the bottom of the pit undergo the greatest modification. Fig. 17 is the central ganglion cell of the ommatidium, with a few slender epithelial cells around it. Fig. 18 is the retinula cell with a few pigmented epithelial cells clinging to its side. Both differ from the rest of the epithelial cells in their possession of nerve processes. In some epithelial cells, as in Figs. 28 and 25, Pl. XXXI, the degree of attenuation is so very great that the protoplasmic character of

the cell body is hardly recognizable. The cell body is represented by an extremely slender, bristle-like filament, with no pigment granules in the inside. In others this hyaline cell body is divided into a number of branches, as shown in Figs. 21 and 24, Pl. XXXI. In other ones, one-half of the cell body is reduced to a hyaline filament, while the other half retains a protoplasmic nature, with a greater or less amount of pigment granules as in Figs. 19, 20 and 24, Pl. XXXI; while in still others the protoplasmic substance is mostly gathered around the nuclei and the two extremities reduced to hyaline structures with no pigment granules in them, as in Figs. 22, 24, 27, 29 and 30, Pl. XXXI.

The contents of the ommatidium of *Limulus* may be put, then, under two categories: (1) the epithelial cells; (2) the products of secretion of these cells. The first may be classified into two principal divisions: (*a*) the neuro-epithelium, (*b*) the ordinary epithelium. The neuro-epithelium is of two kinds, the *central, ganglion cell* and the *peripheral, retinula cells*. The ordinary epithelium shows all varieties of modification, and exists either in the interspaces of the neuro-epithelium or around it. The products of secretion may also be put under two heads: (*a*) the chitin secreted by the neuro-epithelium (the retinula cells) forming the rhabdom; (*b*) the chitin secreted by the ordinary epithelium forming the lens-cone, and the entire chitinous structure lying over the pit.

The basement membrane (*B. M*, Fig. 6, Pl. XXIX) underlies the whole ommatidium, separating it completely from the underlying mesodermic tissue (*Ms*, Fig. 6, Pl. XXIX). At the bottom of the ommatidium the basement membrane follows the course of nerve fibres issuing from the bases of the neuro-epithelial cells, and thus forms a complete sac to the bundle of nerve fibres. When the bundle breaks up in the plexus the basement membrane sheath of the optic nerve fibres becomes indistinct.

A study of transverse sections of the ommatidium at different heights will show the relative positions of the component elements a little more clearly. In Fig. 6*a*, Pl. XXIX, transverse sections of the ommatidium cut at four different levels are shown, and will be intelligible if one compares them with Fig. 6, Pl. XXIX.

In (*a*) Fig. 6*a*, the plane of section passes near the opening of the pit. In the centre is found a portion of the lens (*L*), and

surrounding it a circular row of columnar pigmented epithelium. The lens is secreted by the surrounding columnar cells. Around the whole ommatidium there exists a rather thick, well defined basement membrane (*B. M*) which separates the ommatidium from the mesodermic tissues (*Ms*).

In (*b*) Fig. 6*a*, a section at about the level of the letters *Rt*, in Fig. 6, passing through the sensory portion is shown. The main contents of the ommatidial capsule (*B. M*) are the retinula cells (*Rt*). The axial moiety of each retinula cell is free from the pigment granules, and a thin cuticular covering is secreted around this portion of the cell. This cuticular covering constitutes the rhabdomere (*Rb*). Sections of a number of pigmented cells are seen lying between the retinulae and the basement membrane; these are sections of the perineural epithelial cells.

In (*c*), Fig. 6*a*, a section passing through the body of the ganglion cell (*G*) is shown; the ganglion cell is surrounded by a circle of retinula cells, which are now gradually tapering, each into a slender nerve process. The ganglion cell takes a more eccentric position in (*d*) than in (*c*). The retinula cells (*Rt*) in (*c*) and (*d*) are represented by the circle of nerve fibres surrounding the ganglion cell (*G*).

In (*e*), Fig. 6*a*, the section passes through the bottom of the ommatidium; all of the sensory cells are reduced into nerve fibres which proceed one from the bottom of each cell. The basement membrane (*B. M*) is still distinctly seen, forming the sheath of the nerve bundle. The interspaces of the adjacent ommatidia are occupied by the mesodermic connective tissue.

If we plot out the arrangement of the component elements in the ommatidium of *Limulus*, the appearance shown in Fig. 7 will be presented. The epithelial cells (*Ep*) forming the walls of the pit are drawn with their chitin-secreting surfaces turned towards the axis of the ommatidium, which is morphologically the exterior surface of the body.

c. The Development of the Compound Eye.

The first rudiments of the compound eye appear extremely early in the embryonic stage as a pair of ectodermal thickenings above the level of the "dorsal organ." In Fig. 39, Pl. XXXII, * shows the place where the compound eye makes its

appearance. In this stage the "dorsal organ" (*D. O*) is already very prominent. The eye itself does not show its characteristic invagination, but is represented by a slightly thickened patch of the ectoderm; except in the microscopic examination of the sections it is hardly recognizable. The section Fig. 42, Pl. XXXII, shows an embryo considerably more advanced than Fig. 39. The plane of section passes obliquely forward from behind, and cuts both the eye (*E*) and the "dorsal organ" (*D. O*). The most noticeable feature in this stage of the development of the eye is the existence of the lateral invaginations at the edges of the ocular area. The section shows three portions, the dorsal fold (*d. f*) and ventral fold (*v. f*), with the median portion (*E*) between them. The groove along which the ectoderm invaginates is V-shaped, with the pointed end turned towards the posterior end of the body. As the invagination becomes deeper, the pointed end of the V becomes prolonged into a tube, which works its way beneath the general surface of the ectoderm. This tubular invagination of the posterior part of the eye will be called the median fold of involution (*m. f*) in our subsequent descriptions.

The whole outline of the invaginated area may be represented by Y, the area intercepted by the two divergent branches of the Y being the ommatidial portion, while the posterior stem of the Y is the continuation of the lateral depressions, the median fold, extending backward and downward beneath the surface of the general ectoderm.

The opening of the tube is at the junction of the two divergent branches of the Y with the posterior median stem.

Figs. 47–56, Pl. XXXIII, represent a series of transverse sections of the compound eye in the stage represented by Fig. 42, Pl. XXXII. Fig. 47 is the section passing through the anterior end of the ocular area. The ectodermic cells are considerably thicker than the general ectoderm in this region. The section which comes immediately behind this is not given in the plate. Fig. 48 shows essential characters of the section which immediately follows the section Fig. 47. At two portions of the thickened ectoderm, *d. f* and *v. f*, the cells arrange themselves in radial fashion, accompanied by a characteristic change in each nucleus, which becomes coarsely granular and peculiarly translucent.

In examining the next section, Fig. 49, the radial arrangement of cells in the above section is shown to be the beginning of invagination. In Fig. 49 the lateral invaginations *d. f* and *v. f* have each a well developed lumen. In the median side the walls of the invaginations become continuous with the group of finely divided cells, each with darkly stained nucleus. This group of cells give rise to ommatidia. The outer wall of each invagination becomes continuous with the columnal epithelial covering of the body.

Figs. 50 and 51 show essentially the same thing. The ommatidial area (*Om*) in Fig. 51 becomes wider than in any of the preceding sections.

Just at the point where the maximum width of the ommatidial area is reached (Fig. 51), it again begins to become narrow, for in the section (Fig. 52) which comes immediately behind that shown in (Fig. 51), the ommatidial area is narrower compared with that shown in Fig. 51, and the two lateral folds of invaginations approach each other, resulting in a fusion in the median line in Fig. 53. In Fig. 54 the fused lateral folds, resulting in the formation of the median fold (*m. f*), begins to work its way beneath the level of the skin. In Fig. 55 a few cells (*m. f*), forming the posterior extremity of the median fold beneath the skin, are found. The section (Fig. 56) passing behind that shown in Fig. 55 shows no trace of invagination of any kind.

The anterior end of the eye, then, begins with Fig. 47; two sections behind it a beginning of lateral invaginations becomes discernible; at Fig. 53 the two lateral folds fuse, and at the ninth section (Fig. 54) they form a median tube (*m. f*), and at Fig. 56 the posterior boundary of the ommatidial area terminates.

In Figs. 45*a* and 46*a* are shown the longitudinal sections of the eye. The ommatidial cells in the anterior portion of the eye send out nerve fibres which form the optic nerve (*Op. n*). The basement membrane (*B. M*) which underlies the ectodermal cells forms a complete sheath to the bundle of optic nerve fibres going to the optic ganglia of the brain, which are formed by independent involutions on the ventral side of the body.[1] The origin

[1] The optic ganglia arise as ectodermic involutions in the pre-oral region of the animal. Owing to the existence of a great cephalic flexure in *Limulus*, that side where the optic ganglia as well as the median eye originate has for the sake of convenience been called the "ventral side."

of the optic nerve fibres is further shown in Fig. 43, Pl. XXXII. The figure shows an oblique transverse section through the ommatidial region of a more advanced embryo than the one shown in Fig. 45*a*, Pl. XXXIII. The plane of section passes through the ommatidial area and through the bundle of the optic nerves, which run forward. The migration of the ectodermal cells seems to take place from the periphery to the inside of the body. The basement membrane (*B. M*) forms a continuous sheath around this migratory stream of ectodermal cells.

In Fig. 40, Pl. XXXII, is shown an embryo in the so-called "trilobite stage." A pair of round protuberant organs (*D. O*) in front of the eyes (*E*) are the "dorsal organs." The eye is distinctly marked in this stage, owing to the formation of pigment granules in the component cells. Except in the general enlargement of the organ and the formation of the pigment granules in the cells, the eye in this stage does not offer any material difference from the condition seen in the stage which we have already examined (Figs. 47–56). After the first moult of the chitinous covering from the condition represented in Fig. 40, Pl. XXXII, it passes into a more advanced condition when the essential Limuloid features characteristic of the adult become distinctly discernible. Fig. 41, Pl. XXXII, represents the young *Limulus* at its first stage in the acquisition of the external Limuloid features. The whole body is translucent; the chitinous covering of the body is yet extremely thin, and the dendritic ramifications of the "liver" containing the remnant of the food-yolk of various hues, sometimes green, at other times yellow or pink, shine through the transparent tissues of the body.

The compound eye (*E*) shows an interesting feature in connection with the formation of the ommatidial area.

Figs. 57–64, Pl. XXXIV, show a series of transverse sections through the compound eye in the stage shown in Fig. 41, Pl. XXXII.

Fig. 57, Pl. XXXIV, represents a section passing through the anterior part of the eye. The ectoderm in the ommatidial area is thrown into a number of folds. The cells lying in the valley of the fold are destined to give rise to the ommatidium. In the dorsal margin of the ommatidial area there exists a thick layer of ectoderm, consisting of a number of large cells, each with

granular protoplasm and a large nucleus. At the basal end of each cell there exists an accumulation of transparent liquid substance. Beneath ommatidial area a well defined basement membrane (*B. M*), which is the continuation of the same membrane existing under the general ectoderm of the body, sharply marks out the ommatidial structure from the mesodermic tissues.

In Fig. 58, which is taken a few sections behind the preceding, is shown the ommatidial area at its widest part. Four ommatidia formed by the undulating folds of the ectoderm are shown, each depression being accompanied by a corresponding thickening of the chitin, forming the rudiment of the lens-cone (*C*).

On both sides of the ommatidial area we find the lateral invagination of the ectoderm. The cells forming the invaginated folds undergo enormous enlargement, and in the basal end of each cell is shown a large accumulation of transparent fluid. The cells forming the inner walls of the dorsal and ventral folds of invaginations undergo various metamorphoses. Each of them becomes divided into a number of smaller cells, each of these segments being packed with extremely fine pigment granules. As the cells in the inner walls of the dorsal and ventral folds (*d.f*, *v.f*) become divided up into a number of smaller pigmented segments, they are constantly pushed out towards the surface, where they, grouping themselves into a number of ommatidia, increase the general surface of the ommatidial area. Thus, the older ommatidia are found in the middle and the younger ommatidia are to be found around the marginal portion of the ocular area.

In Fig. 59 a section is shown in which the ommatidial area is narrower than the preceding. In Figs. 60 and 61 this narrowing of the ommatidial portion continues, and in Fig. 62 the dorsal and the ventral folds meet in the median line. In Fig. 63 the median fold becomes completely buried beneath the general ectoderm. In Fig. 64 the section passes near the posterior extremity of the median fold, where the reduction in the size of the tube as well as its increased distance from the level of the general ectoderm is clearly discernible.

In all the figures above described *Pg. c* means the pigment cells, which in the state of nature are highly pigmented, each with a small eccentric nucleus. Each section has been carefully

depigmented, without which the process of the formation of the ommatidia and the increase of the ocular area is perfectly unintelligible. Those cells in the figures which have no granular structure are, in the state of nature, charged with a large quantity of pigment granules, and those large cells forming the walls of the lateral folds are granular and free from pigment granules.

Transverse sections are not fitted for the study of the formation of the nerve fibres. In this stage, as in the preceding ones, each ommatidium sends out nerve fibres from its basal end towards the interior of the body. Besides the cells in the bottom of each ommatidial pit, each gigantic cell of the outer walls of the dorsal and ventral folds sends out a nerve process from its basal end, which runs inward and forward, joins the nerve fibres from the bottom of each ommatidium, forming a large bundle, and goes to the brain.

The invagination of the lateral and posterior margin of the ocular area is usually not permanent. With a growth of the animal and an increase of the surface of the ocular area, the invaginated folds, both on the sides as well as behind the ocular area, become stretched out in the adult animals and leave no trace of their existence. Occasionally, however, one meets in the adult with a remnant of the involuted tube in the posterior part of the eye, which exists as a ball of pigment cells buried in the midst of the mesodermic tissue.

The history of the ommatidial cells in the compound eye of *Limulus* may then be stated in the following way:

(1). A stage of undifferentiated ectodermal cells.

(2). Thickening of these undifferentiated cells, accompanied with the invagination beneath the surface.

(3). Extreme enlargement of the invaginated ectoderm, followed by the acquisition of pigment granules and by division.

(4). Pushing out of the resulting small pigmented cells towards the surface where they differentiated into two fundamental groups: (*a*) Ordinary epithelial cells, which secrete the chitinous cuticle over the ocular area, including the lens, or pack the interspaces of the neuro-epithelium; (*b*) neuro-epithelium, consisting of the rod-bearing retinulae and the central ganglion cells, the two forming the sensory portion of the ommatidium.

IV.—Phylogeny of the Ommatidium.

I do not pretend to enter into a detailed discussion of this difficult problem. In order, however, to give a more intelligent idea of what I have thus far been considering, I will devote the following pages to the reconsideration of facts given in the preceding pages from a more comprehensive standpoint.

Balfour[1] has given a sketch of the possible evolution of a visual organ. He starts with a simple organism in which a spot on the surface of the body may become spontaneously pigmented and therefore become specially sensitive to light. The cuticular covering of the body may become thickened at this spot and act as an apparatus for condensing the light upon the pigmented spot lying beneath it. He further expresses his view elsewhere[2] that the lens-like dioptric apparatus of the eye, formed either as a thickening of the cuticle or as a mass of cells, was at first formed simply to concentrate the light on the sensitive spot; the power to throw an image of external objects on the perceptive part of the eye was acquired gradually afterward.

The part which is played by pigment in the physiology of vision is considered a most obscure problem. I quote the following, clearly put forward by Foster,[3] as a physiological aspect of the question bearing upon the discussion at issue: "But in order that light may produce chemical effects (upon protoplasm), it must be absorbed; it must be spent in doing the chemical work. Accordingly, the first step towards the formation of an organ of vision is the differentiation of a portion of protoplasm into a pigment at once capable of absorbing light and sensitive to light—*i. e.* undergoing decomposition upon exposure to light. An organism, a portion of whose protoplasm had thus become differentiated into such a pigment, would be able to react towards light. The light falling on the organism would be in part absorbed by the pigment, and the rays thus absorbed would produce a chemical action and set free chemical

[1] F. M. Balfour: Address to the Department of Anatomy and Physiology, British Association, 1880; *Nature*, Vol. XXII, p. 417, 1880.

[2] *Comparative Embryology*, Vol. II, Chapter XVI, Organs of Vision, p. 470.

[3] M. Foster: *A Text Book of Physiology*, 4th Edition, Book III, Chap. II: Sight; The Photochemistry of Retina, pp. 515–516.

substances which before were not present. We have only to suppose that the chemical substances are of such a nature as to act as a stimulus to the protoplasm of other parts of the organism (and we have manifold evidence of the exquisite sensitiveness of protoplasm in general to chemical stimuli), in order to see how rays of light falling on the organism might excite movements in it, or modify movements which were being carried on, or might otherwise affect the organism in whole or in part. Such considerations as the foregoing may be applied to even the complex organ of vision of the higher animals. If we suppose that the actual terminations of the optic nerve are surrounded by substances sensitive to light, then it becomes easy to imagine how light, falling on these sensitive substances, should set free chemical bodies possessed of the property of acting as stimuli to the actual nerve-endings, and thus give rise to visual impulses in the optic fibres."

Lubbock[1] advances essentially the same idea as Balfour's in his recent work on the subject, illustrated with some lucid diagrams. "In the simple forms," he says, "the whole surface is more or less sensitive. Suppose, however, some solid and opaque particles of pigment deposited in certain cells of the skin. Their opacity would arrest and absorb the light, thus increasing its effect, while their solidity would enhance the effect of external stimulus. A further step might be a depression in the skin at this point, which would serve somewhat to protect these differentiated and more sensitive cells, while the deeper this depression the greater would be the protection."

That such steps of gradual development of visual organs have actually taken place in some forms is quite probable. In Arthropods, it seems to me worthy of remark that the ommatidium of the lateral eye of *Limulus* makes the nearest approach to this primitive condition. It is nothing more and nothing less than a depression in the skin, with the thickened chitinous cuticle fitting in the open cavity and acting as a lens to condense the light. The cells which form the sensory part of the structure are modified ectodermic cells, and, like the rest of the ectodermic cells lying on the surface of the body, secrete the chitinous cuticle on

[1] *On the Senses, Instinct, and Intelligence of Animals*, Inter. Scie. Series, Vol. LXIX, 1888.

a part of their surface. Assuming the surface where the chitin is secreted to be the exterior, we may describe the ommatidium of the lateral eye of *Limulus* as a group of modified ectodermic cells aggregated around and beneath the funnel-shaped depression in the skin. A glance at the diagrams (Fig. 6, Pl. XXIX; Figs. 65 and 66, Pl. XXXV) will show this clearly. The sensory cells of the ommatidium come into direct contact with the conical lens, which is the thickened part of the general cuticle; or, to express this in the phraseology of Lankester and Bourne, the ommatidium in the lateral eye of *Limulus* is "epistatic." The cornea and crystalline cone as such have no separate existence in this stage.

Suppose such an ommatidium to become duplicated until a considerable number be formed, as we may safely imagine to have been the case, from the general tendency in the perfection of a visual organ. What will be the result? The first effect of such an increase of the number of ommatidia in a given area will be the lengthening of each unit in the direction of the ommatidial axis, and the cells (*V*, Fig. 67, Pl. XXXV) which were situated directly on the outside of the retinulae will travel over and above the sensory portion (*Rt* and *G*, Fig. 67). The distal ends of such cells (*V*) which were thus pushed over will meet one another in the median or the "optic axis" of the ommatidium; further, they will continue to secrete chitin (*c. c*, Fig. 67) from their original chitin-secreting surfaces which are now median and axial. The chitin thus secreted will have an independent existence from the cornea, thus forming the rudiment of the crystalline cone, and the cells themselves will form the vitrellae (*V*, Figs. 67, 68, etc.). Finally, as the deepening still further goes on, the corneal lens (*C*) and crystalline cone (*c. c*) will be entirely separated, thus producing a condition somewhat similar to that which obtains in *Serolis* (Figs. 69 and 70.)

From this point onward, the three chitinous structures, *cornea*, *crystalline cone*, and *rhabdomere*, undergo a different development in different Arthropods. In some the crystalline cone assumes a transparent semi-liquid state, while the whole cell becomes extremely elongated, forming the crystalline cone of certain crustacea (Fig. 71, Pl. XXXV); it may form a hard

chitinous ball as in *Serolis* (Fig. 70, Pl. XXXV); or a cuneiform chitinous structure, as in *Talorchestia* (Fig. 73, Pl. XXXV); or finally the whole cell may remain as a clear, transparent body, as in several insects, forming Grenacher's "aconous type" of the compound eye.

The forms assumed by the rhabdomeres in different Arthropods are equally diverse. The rhabdomere may exist as a plain cuticular covering over the non-pigmented part of the retinula, as in *Limulus* or in *Serolis* (Fig. 10, *Rb*, Pl. XXIX; Fig. 38, *Rb*, Pl. XXXI, etc.); it may become extremely elongated and narrow as in *Musca* or in *Callinectes* (Fig. 5, *Rb*, Pl. XXIX; Fig. 37, *Rb*, Pl. XXXI; Fig. 72, *Rb*, Pl. XXXV); it may become transversely folded as in *Cambarus* (Fig. 35, *Rb*, Pl. XXXI; Fig. 4, *Rb*, Pl. XXIX; Fig. 71, *Rb*, Pl. XXXV); these transverse folds may become still finer, showing the chitinous serrature along the axial edge of the retinula, as in *Penaeus* and *Homarus* (Fig. 34, *Rb*, Pl. XXXI); or this transverse serrature may become extremely fine and regular, as in *Squilla* (Fig. 36, Pl. XXXI).

The cornea undergoes equally diverse modifications according as it is purely protective, or partly protective and partly dioptric in function. The range of variation is shown by the degrees of curvatures and by the varieties of its thickness. In several of the decapod crustacea which I have examined, as *Penaeus*, *Cambarus*, *Homarus*, *Callinectes*, *Gebia*, etc., the curvatures of the individual cornea on both surfaces are very slight; it is biconvex in an extremely small degree. In *Talorchestia* both surfaces of the cornea are parallel. In *Serolis*, four species of which I have studied, all having well developed compound eyes, there exists a considerable difference in different species in the nature of the cornea. In some the curvature on the proximal surface is very strong and the whole structure is quite thick, while in others the cornea is rather thin and a slight development of curvatures exists. This is interesting, showing that even within the group of nearly allied species there are considerable differences in this respect.

This fact is easy to understand when we remember the functional property of the cornea and the crystalline cone. As has been noticed already, the crystalline cone is always dioptric in

function, while the cornea may be partly protective and partly dioptric, or wholly protective. When the cornea becomes partly dioptric, as in *Serolis* and in several other Arthropods, the dioptric function in an individual ommatidium comes to be performed by two structures, the crystalline cone and the corneal lens. When the two structures act together for the same end at the same time, it is easy to see how a certain trivial peculiarity of the one may induce a correlative modification of the other, and how a slight specific peculiarity may appear exaggerated in the thickness or in the degree of curvature of the corneal lenses in different species.

After so much has been said in regard to the unity of structure of the ommatidium in different Arthropods, one important point awaits our consideration, viz. the homology and fate of the central ganglion cell found in the ommatidium of *Limulus*. Unless a great many forms of ommatidia in different Arthropods be compared, a discussion on this point appears to be unprofitable. The consideration which follows is therefore a purely provisional one.

There can be no question that the central ganglion cell is an important factor in the ommatidium of *Limulus*, nor can we doubt the existence of a fundamental homology between the retinulae of *Limulus* and those of all the other Arthropods which I have examined. With the exception of a few problematical bodies, such as the "hyaline cells" of *Serolis*, there are no structures in the ommatidia of most Arthropods which correspond to the central ganglion cell of *Limulus*, in spite of the existence of a fundamental homology in the other elements of the ommatidium.

What has become of the central ganglionic element of the ommatidium? Was it lost in the course of the phylogenetic history of a more complex ommatidium? Or is it reasonable to suppose that some ommatidia came into existence without it from the beginning? Or, if it were lost at all, is there any evidence which makes this supposition probable?

The colorless ganglionic cell and the pigmented rod-bearing cells which surround the former I consider as the two primitive morphological factors in the unit of the sensory part of the Arthropod retina, somewhat in the same way as the circle of

rods with a cone in the centre are the two essential factors in the neuro-epithelial layer of the human retina. In the absence of enough comparative data in Arthropods at present we have to dwell largely on the analogy suggested in the other groups of animals. Whatever be the views as to the fundamental homology of the ommatidium of *Limulus* to a structural unit of the sensory part of the human retina, a superficial resemblance of the one to the other is certainly very strong. The structural resemblance is paralleled by a physiological one. The place where the light acts in the visual end-organ of Arthropods and of man may alike be considered as consisting of a number of definite groups of cells, each group being a morphological and a physiological unit; or, in other words, the sensory part of the retinæ in both cases consists of a mosaic of several sensitive spots. The image formed on such a surface is therefore a mosaic one, whether in an Arthropod or in a Vertebrate.

Fundamental as this arrangement appears to be in the human retina, these two factors are liable to variation in their relative distribution in different Vertebrates. In fact, the variation takes place between the two extremes where the rods alone exist on one hand and where the cones alone constitute the essential part of the retina on the other. Thus, according to Schultze, "either form of percipient element (rod and cone) may be represented by the other" in the Vertebrate. This range of variability in the distribution of the cones and rods occurs even in a single group of Vertebrates, as in mammalia, showing that the variation in the distribution of the essential factors, even within a tolerably well circumscribed group of animals, is sometimes quite extensive. The group of Arthropods is a heterogeneous one, and I see no *à priori* objection to believing in the existence of a phenomenon analogous to what we find in Vertebrates, viz. that the two percipient elements represented by the central and the peripheral cells in the ommatidium of *Limulus* may be differently represented in different Arthropods.

There is no doubt whatever that the retinula cells are homologous throughout the Arthropods. In fact, in most Arthropods which I have examined no other elements but the retinulae have any connection with the optic nerve fibres, and they often undergo an enormous development and acquire most complicated

structures, as in *Homarus* or in *Penaeus*, giving rise to the much discussed "spindle."

But what has become of the central element which is so conspicuous in the ommatidium of *Limulus*, if the retinulae in all Arthropods are homologous? I believe the central cell is fully functional, judging from its position and from its veritable connection with optic nerve fibre in *Limulus*. What in other Arthropods strongly reminds one of this cell is the "hyaline cell" at the bottom of the ommatidial pit in *Serolis* and, according to Beddard, also in the *Cymothoidae*. One important difference, however, exists between the "hyaline cell" of the Isopods and the central cell in the ommatidium of *Limulus*, viz. that, while in the latter the cell is connected with the optic nerve, the "hyaline cell" in the former has no connection with the central nervous system whatever. Hence the "hyaline cell" cannot be sensory, even if it be homologous with the central cell of *Limulus*, which it resembles in its general appearance and in its position. The number of "hyaline cells" in *Serolis* is always two, while its supposed homologue in *Limulus* is, as a general rule, only one. This fact does not offer any objection to my view of their homology, when we bear in mind that other elements in different ommatidia, as vitrellae and retinulae, show a wide range of variation so far as their numbers are concerned, and yet they can be considered as perfectly homologous.

A further embryological and comparative knowledge in regard to the "hyaline cell" in Isopods is necessary for the determination of its exact homology. Meanwhile I would observe that if the central and the peripheral cells which we see in the ommatidium of *Limulus* may be taken as the two essential factors of the sensory element of the typical Arthropod retina, the case of *Serolis* may be taken as a loss of balance in the relative development of these two factors; the central cells having lost their sensory function and remaining as a sort of supporting mechanism. We can imagine this change in the function of the central cell as carried still further, and with the excessive development of the peripheral elements, the retinulae, the central element may finally have disappeared.

All this is, however, a mere suggestion, and my interpretation of the nature of the Arthropod ommatidium in general does not

lose its force even if this section of my views in regard to the fate and homology of the central cell or cells be proved untenable. It is quite possible that the ommatidia in which there is no element corresponding to the central cell of *Limulus* may have originated without it from the beginning. It seems, however, more natural to suppose that such an ommatidium had it originally and lost it later, observing that the simplest form of ommatidium possesses it in its fully functional, sensory form.

Finally we have to consider the nature of the compound eye as a whole as presented in various types of Arthropods.

That a certain structure in the body of an animal may repeat itself and give rise to a secondary aggregate, or to a compound organ, is a well known fact; the repetition of similarly constructed uriniferous tubules forms the essential part of a vertebrate kidney, or the similar repetition of gill-filaments forms the respiratory organ of a Lamellibranch. Sundry other examples of this nature might be given, but the above two will suffice. Tracing, as I have attempted to do, the most complicated ommatidium into a simple, open ectodermic pit, there is to my mind no difficulty in believing that the compound eye of the Arthropod is one of the most astonishing examples of the formation of an organ by the vegetative repetition of the similar structure. Thus, according to Lubbock, there are about 4000 facets in the compound eye of the house-fly (*Musca*), each facet corresponding to a single tubular invagination of the skin, the ommatidium. There are 4000 independent invaginations in the area in the head of the fly occupied by the compound eye; in the gadfly (*Œstrus*) 7000; in the goat-moth (*Cossus*) 11,000; in the death's-head moth (*Sphinx atropos*) 12,000; in a butterfly (*Papillio*) 17,000; in a dragon fly (*Æschna*) 20,000; in a small beetle (*Mordella*) as many as 25,000. On the other hand, the number of ommatidia seems to have reached its minimum in certain Copepods, as in *Corycœus*, where the whole visual organ seems to be represented by a single colossal ommatidium.

Certain forms of Collembola[1] seem to have a very small num-

[1] Lubbock. *Monograph of the Collembola and Thysanura*, the Ray Society, 1873, p. 57, Pls. LV and LVI. Lubbock uses the term "ocellus" to designate a single element of the eye which I here called an ommatidium. If the structure of this "ocellus" differs from the ommatidium of other Arthropods, it has, of course, nothing to do with the discussion at issue.

ber of ommatidia; thus in *Templetonia* only one ommatidium exists on each side of the head; *Orchesella* has six on each side of the head; *Tomocerus*, *Isopoma*, have seven; *Degeeria*, *Lepidocyrtus*, *Smynthurus* and *Papirius*, eight. In the ants we observe a similar gradation in the number of ommatidia.

What reasons can we assign for this enormous multiplication of similarly constructed parts? What advantage follows from this arrangement? If the view of the nature of the compound eye which is put forward in the preceding pages be a true one, Müller's celebrated theory of mosaic vision is the only one that can account for the enormous multiplication of the similarly formed pits in the skin. The subject has been so fully discussed by Lubbock that I need not enter into details here. "According to his (Johannes Müller's) view, those rays of light only which pass directly through the crystalline cones, or are reflected from their sides, reach the corresponding nerve fibre. The others fall on and are absorbed by the pigment which separates the different facets. Hence each cone receives light only from a very small portion of the field of vision, and the rays so received are collected into one spot of light. *The larger and more convex, therefore, is the eye, the wider will be its field of vision; while the smaller and more numerous are the facets, the more distinct will the vision be.* In fact, the picture perceived by the insects will be mosaic, in which the number of points will correspond with the number of facets."[1] The whole explanation of the problem seems to me to be contained in the passage above cited; and no further comment will be necessary more than a statement that the increase in the number of ommatidia is a decided advantage to their possessor. An eye like that of *Limulus* might by a slight change be converted into one of a more protuberant nature so as to command a wider field of vision, as we see in some species of *Serolis* or in some Trilobites; a slight change again might produce a protuberant ocular area mounted on an ophthalamic stalk, and accompanied by the accessory apparatus of vision, such as the socket for protection or the set of muscles to move the eye-stalk in different directions so as to command a still wider field of vision. In this connection I may refer to a series of diagrams (Figs. 74–81, Pl. XXXV). The black heavy layer repre-

[1] Lubbock: *Senses, Instinct and Intelligence*, p. 163.

sents the ectoderm, and the region in which the ectoderm is thrown into folds the area of the compound eye. The yellow colored layer outside represents the chitin, and the dotted line beneath the ectoderm, the basement membrane.

In *Limulus* (Fig. 74) the ectoderm is thrown into a series of shallow folds, which, when viewed from above, would be a group of shallow pits in the skin. Each pit is an ommatidium. In *Serolis* (Fig. 75) the invagination of the skin is a little deeper than that of *Limulus*, and the whole ocular area is more prominent. Fig. 76 represents the condition of the ectodermal folding in *Notonecta*, and Fig. 77 that of *Agrion* larva. Fig. 78 represents the eye of *Branchipus*, only a part of the stalk being shown in the figure. Fig. 79 represents the eye of *Cambarus;* Fig. 80 that of *Penaeus*, and Fig. 81 that of *Lucifer*.

It must not be understood that the number of folds given in the diagrams have anything to do with the actual number of ommatidia that may exist in the actual specimens; no more than a morphological expression of the eye in a simplest possible form was intended. If one suppose a single invagination of the skin, say of Fig. 79, be divided into three strata and the cells in the bottom stratum to send out nerve fibres, those in the middle to form the crystalline cone and those in the outermost to form the cornea (Fig. 71), the interpretation of the diagram will be complete.

According to this view the compound eyes of Arthropods, either in the sessile or in the stalked form, are nothing more than a collection of ectodermic pits whose outer open ends face towards the sources of light, and whose inner ends are connected with the central nervous system by the optic nerve fibres. The cells forming the walls of the pit arrange themselves into three strata, in most cases accompanied by three regional functional differentiations. Grenacher's classification of the compound eyes of insects into "acone," "pseudocone" and "eucone" types refers to the condition of the cells and their products in the middle stratum—the vitrellae.

Morphologically, then, the compound eye of an Arthropod is strictly single-layered, although, as is evident, the present conception is entirely different from the monostichous theory maintained by some recent writers. From *Limulus* to *Squilla* we

have a series of forms showing all degrees of modification in the general structure of the eye as well as the structure of its individual elements, and there is not here a single form which invalidates the view maintained in the present paper. Moreover, this view has the advantage of greatly simplifying our conception of these structures, reducing, as it does, all of them to one primitive structure, a depression in the skin, in which several organs of ectodermal nature, often of a very complicated type, find their common morphological origin. And when thus the nature of the unit is reduced into a simple invagination of the skin, the formation of the compound eye appears to be but another instance of the well known method in the formation of a morphological organ, namely, *the vegetative repetition of a similar structure.*

V.—Summary.

In studying the structure of the ommatidium of the compound eye of *Serolis* it has been found that it may be reduced to a simple ectodermic invagination of the skin. Extending my researches over several other Arthropods, of which *Talorchestia*, *Cambarus*, *Homarus*, and *Callinectes* were mentioned in the preceding pages, the same interpretation of the ommatidium may be applied without exception. This view of the ommatidium finds its strongest support in the fact that in *Limulus*, the ommatidium is an open pit of the skin.

By supposing that the ommatidial pit of *Limulus* became deeper and that this was accompanied by modifications in the structure and arrangement of the component cells, we can show the probability of our first supposition that the ommatidium of the compound eye of an Arthropod is an independent invagination of the skin. If this view is correct, the unit of the compound eye of an Arthropod is not, after all, so complex a structure as has been supposed by some; and the enormous increase in the number of ommatidia in a given area of the skin which results in the formation of the compound eye finds its parallel in the well known method of the formation of morphological organs, viz. the duplication of a simple unit.

Baltimore, *April* 30, 1889.

VI.—Appendix.

The Compound Eye of Echinoderms.

While the preceding paper on the compound eye of Arthropods was passing through the press I improved my opportunity to study the compound eye of Echinoderms at Woods Holl, during the months of June and July, 1889, to see how far the morphological interpretation advanced for the former will hold to the latter.

The three starfishes common at Woods Holl, viz. *Asterias vulgaris*, Stimp., *Asterias forbesii*, Verril, and *Cribella sanguinolenta*, Lutken, were studied in this connection. While I reserve the description of the details for a future paper, I will briefly point out some important bearings of the results upon the subject which has been discussed in the preceding pages.

The general structure of the compound eye of the starfish at the tip of each ray is well known through the writings of Hæckel, Wilson, Hoffmann, Lange, Greeff, Hamann, Carrière, and some others, although there exist some discrepancies in regard to the histological features of the organ as described by these naturalists.

The most remarkable observation on the eye of the Echinoderm among the early writers is that of Goodsir, who, according to Forbes,[1] is said to have examined the eye of *Cribella oculata* and remarked of its structure that it consisted "*of a red cushion with pits on its surface.*" My studies on the compound eyes of the starfishes convinced me of the truth of Goodsir's statement, and the eye of a starfish is nothing more than a group of ectodermal conical pits with their bases turned towards the exterior. The epithelial cells which form the walls of the conical pit have each a cuticular rod secreted at the tip of its distal extremity.

These cells send out nerve fibres from their proximal extremities. In the centre of the pit along the longitudinal axis of the invagination there is a space filled with a clear fluid substance. The cuticular secretions of the retinal cells, as well as the colorless fluid substance, constitute together a translucent

[1] E. Forbes: *A History of British Starfishes, and other Animals of the class Echinodermata.* London, 1841, p. 102.

refractive body in the centre of each pit or the ommatidium, which has been described by several as the "crystalline cone" in the starfish eye. The whole surface of the eye-bulb is covered by a thin cuticle which is secreted by the cells whose distal extremities lie on the general level of the external surface of the optic bulb. In short, this external cuticular covering of the eye in a starfish corresponds to the *cornea*, and the cuticular secretions at the distal extremities of the retinal cells forming the walls of the pit, correspond to the *crystalline cone* and the *rhabdom* of the compound eye of an Arthropod. The colorless fluid substance contained in the axial space of the invagination in the starfish may be compared to the similar substance in the crystalline cone of the ommatidium of *Serolis*. The number of ommatidia in the starfish increases at the periphery of the optic tract as in an Arthropod. The spaces between the adjacent ommatidial invaginations are occupied by the supporting epithelial cells. The optic nerve fibres pass into the deepest stratum of the epithelial layer, and running horizontally, join the main branch of the ventral nerve cord of the ray.

On the whole, the entire morphological arrangement of the parts in the compound eye of an Echinoderm is strikingly similar to that of an Arthropod, and the formation of the visual organ in the Echinoderms, at least in one of the large sub-groups, the *Asteridæ*, by a series of ectodermic invaginations, assumes a wider significance when we take into consideration the fact that the compound eye of the Arthropods has a similar mode of origin, although it is an independent adaptation.

In the anatomical consideration of the Echinoderm eye I have made no allusion to the so-called "eye-spot" on the ocular plate of the sea-urchin. In regard to the function of this structure we are by no means certain that it is visual in nature as it is usually assumed. Valentin failed to discover any trace of dioptric apparatus in it. More recently Fredericq[1] could not find any evidence, anatomically and experimentally, that the structure in question is an eye. "*La tache de pigment qu'on y décrit est une pure fiction.*"

[1] Fredericq, Léon: *Contributions à l'étude des Echinides.* Archives des Zoologie Experi. et Gener., Tome 5, 1876, p. 484.

Hamann,[1] who studied this structure in Echinus, declares that the so-called "eye-spot" in the sea-urchin bears a close resemblance to the terminal feeler at the tip of arm of a starfish, with which he thinks it is homologous and not with the eye. He therefore substitutes the term "intergenital plate" for the "ocular plate" upon which this problematical organ rests.

Even if this organ be shown to have some connection with the visual function, as Romanes and Ewart[2] claim on the ground of their experiments, it is safe to say that it belongs to a different category of visual organ, as Hamann's histological study clearly shows, and has nothing to do with the subject under our consideration, viz. the morphology of the *compound eye* of the Echinoderm and the Arthropod.

Entirely apart from the preceding, but which may be mentioned in this connection, is the Sarasins' discovery[3] of the compound eyes in a sea-urchin (*Diadema*) from Ceylon. Of their visual nature there can be no doubt, as their experiments on the living animals as well as the anatomical structure of the eyelet show.

The eyelet of the compound eye in *Diadema* is very much more complex than that of the starfishes I have examined. I cannot help but believe that a study of development of such an interesting form as *Diadema*, as well as a more extensive study of the eye in different genera and species of Echinoderms, will throw a great deal of light on the morphology of the compound eye in general.

In my main paper on the subject I hope to describe in detail the structure and development of the compound eye of the starfish that I have studied, and also to give an account of the growth of our knowledge of the visual organ of the Echinoderms, both anatomical and experimental, from Tiedman and Ehrenberg down to the present day.

[1] Hamann, Otto: *Beiträge zur Histologie der Echinodermen.* Jenaische Zeitschrift für Naturwissenschaft, Bd. 21, 1887, pp. 124–126.

[2] Romanes, J. G., and Ewart, J. C.: *Observations on the Locomotor System of Echinodermata.* Philosophical Transactions, Royal Society, London. Pt. III, 1881, pp. 855–856.

[3] Sarasin, P. B. and C. F.: *Ueber einen mit Zusammengesetzten Augen bedeckten Seeigel.* Zoologischer Anzeiger, 8 Jahrg. No. 211, 1885. Their main paper I have not seen.

VII.—Explanation of Figures.

List of Reference Letters.

Ap. Appendage.
Ax. p. Axial process of the ganglion cell.
B. M. Basement membrane.
C. Corneal lens.
c. Cornea or corneal facet
c. c. Crystalline cone.
Ch. Chitin.
c. g. Corneagen.
d. f. Dorsal fold.
D. O. Dorsal organ.
E. Eye (compound).
Ect. Ectoderm.
Ent. Entosternite.
Ep. Epithelial cell.
F. y. Food-yolk.
G. Ganglion cell.
H. Hyaline cell.
L. Lens-cone.
Ms. Mesoderm.
N. Nucleus.
Oc. Ocellus.
Om. Ommatidium.
Op. n. Optic nerve fibre.
p. c. Packing cell.
p. n. Perineural cell.
Pg. c. Pigment cell.
Pg. p. Pigment patch.
Rb. Rhabdomere.
Rt. Retinula.
Tr. fb. Transverse striae.
V. Vitrella.
V. C. Ventral nerve chain.
v. f. Ventral fold.
x. Axial space or line of the ommatidium, corresponding to the cavity of the invagination.

PLATE XXIX.—*Serolis, Talorchestia, Cambarus, Callinectes* and *Limulus*.

FIG. 1.—*Serolis*. Two ommatidia of *Serolis;* the one on the left with the pigment cells enveloping the crystalline cone. The downward extensions of the rhabdomeres reach to the retinal side of the basement membrane. ½(E × 2) Zeiss.

FIG. 1*a*.—*Serolis*. Diagram of the Ommatidium—

a. Corneagen.
b. Vitrella.
c. Retinula.
d. Hyaline cell.
1. Cornea.
2. Crystalline cone.
3. Rhabdomere.
Pg. c. Pigment cell.

FIG. 1*b*.—*Serolis*. Plan of the Ommatidium—

1. Hyaline cell (*d*).
2. Retinula (*c*).
3. Vitrella (*b*).
4. Corneagen (*a*).
5. Pigment cell (*pg. c*).

FIG. 2.—Morphological diagram of the ommatidium of *Serolis*—

a. Corneagen.
b. Vitrella.
c. Retinula.
d. Hyaline cell.
1. Cornea.
2. Crystalline cone.
3. Rhabdomere.

FIG. 3.—*Talorchestia*. Ommatidium of *Talorchestia*.—*Rt'*, upward extension of the retinula *Rt*; this upward extension sometimes reaches as far as the cornea. *p. c*. Narrow, non-pigmented packing cells. ½(D × 4) Z.

FIG. 3*a*.—*Talorchestia*. Plan of the Ommatidia.

FIG. 4. *Cambarus*. Three ommatidia shown side by side. In *c* the pigment cells (*pg. c*) completely envelop the crystalline cone. *x* is the morphological lumen of the invagination. *Ep* is the epithelial cell charged with light yellow pigment granules. ½(D × 2) Z.

FIG. 5. *Callinectes*. Two ommatidia shown side by side. Rhabdomere (*Rb*) shows a fine striation. At the swollen end of the retinula (*Rt*) yellow or brown colored globules are found.

FIG. 5*a*.—*Callinectes*. Head of the retinulae more highly magnified, and the rhabdomeres show slight enlargement at the terminal portion.

FIG. 6.—*Limulus*. Two ommatidia shown side by side, partly schematic. The thick yellow-colored body is the chitinous covering of the eye. *L*. lens-cone, fitting into the depression of the skin. At the bottom of the depression, the essential part of the ommatidium

is found, consisting of two parts, *Rt* (retinulae) and *G* (central ganglion cell). Cells forming the walls of the pit are the perineural cells (*p. n*). Inward prolongations of the neuro-epithelial cells (*Rt* and *G*) form the optic fibres (*Op. n*). *Ms.* Mesodermic tissue.

FIG. 6*a*.—*Limulus*. Transverse sections of five ommatidia at different levels. In (*a*) the plane of section passes through the upper part of the depression, cutting the lens-cone (*L*); in (*b*) the section plane passes through the retinulae, showing the star-shaped rhabdom and the axial process of the ganglion cell (*Ax. p*) in the axial canal of the rhabdom. A few pigmented perineural cells surround the retinulae, while the basement membrane (*B. M*) forms the complete capsule of the ommatidium, separating the latter from the mesodermic tissue (*Ms*). In (*c*) the plane of section passes through the lower part of the ommatidium, cutting the central ganglion cell (*G*); in (*d*) the ganglion cell occupies an eccentric position; in (*e*) the sections of optic nerve fibres alone are seen.

FIG. 7.—*Limulus*. Plan of the ommatidium. *Ep* represent the cells which are found in the walls of the pit; the yellow edge corresponds to the surface where the chitin is secreted.

PLATE XXX.—*Limulus*.

FIG. 10. Retinula (*Rt*) and ganglion cell (*G*) teased out from the ommatidium after treatment with Haller's fluid 15 hours. The axial process (*Ax. p*) of the ganglion cell closely follows the external border of the rhabdomere (*Rb*). A number of epithelial cells are found closely attached to the surface of the retinula as well as to the ganglion cell. ⅔ (E × 2) Z.

FIG. 11. A bunch of retinulae completely isolated from the ommatidium. Pigmented epithelial cells (*Ep*) are found between them. The proximal ends of retinulae form the optic nerve fibres (*Op. n*). ⅔ (E × 2) Z.

FIG. 12. A retinula cell seen from the side opposite to the rhabdomere. A number of extremely attenuated epithelial cells (*Ep*) are found closely clinging to the external surface of the cell. They do not form any connection with the retinula, and can be completely isolated from it. The cell-body of such an elongated epithelium is reduced to a very slender filament and does not contain any pigment granules. ⅔ (E × 2) Z.

FIG. 13. The central ganglion cell with its axial process (*Ax. p*) completely isolated. The pigment patch (*Pg. p*), situated between

the nucleus (*N*) and the optic nerve fibre (*Op. n*), shows a characteristic mark of the cell. A zone around the nucleus (*N*) shows concentric markings. Longitudinal striae run through the axial process and the optic nerve; (*Ep*) an attenuated epithelial cell. $\frac{2}{3}$ (E × 2) Z.

FIG. 14. Another type of the central ganglion cell. Pigment patch is greatly extended; nucleus (*N*) is eccentric; (*Ep*) attenuated epithelial cells. $\frac{2}{3}$ (E × 2) Z.

FIG. 15. The central ganglion cell, with its axial process (*Ax. p*) divided into several branches, by some mechanical causes. All these branches are longitudinally striated like the main one and would seem to be made up of a collection of extremely fine longitudinal fibrils. Two pieces of chitinous body (*Rb*) are found at the junction of the axial process and the cell-body proper of the ganglion cell. They appear to be fragments of the rhabdomeres. $\frac{2}{3}$ (E × 2) Z.

The specimens figured in Figs. 10–14 were treated with Haller's macerating fluid; that shown in Fig. 15 was treated with sulphuric acid, 8 drops to 30 grams of water.

FIG. 16. Epithelial cells isolated from the margin of the ommatidial depression. The part in which there is no pigment is turned outward and comes in direct apposition to the chitin.

PLATE XXXI.—*Limulus, Homarus, Cambarus, Squilla, Callinectes, Serolis.*

FIGS. 17-33.—*Limulus.* This series of specimens is intended to show the range of modification of the epithelial cells found in the ommatidium and its direct neighborhood.

FIG. 17. Central ganglion cell with several attenuated, non-pigmented epithelial cells. $\frac{2}{3}$ (E × 2) Z.

FIG. 18. Retinula cell with a few pigmented epithelial cells at its side. $\frac{2}{3}$ (E × 2) Z.

FIGS. 19–33. Ordinary epithelial cells isolated from the ommatidial region. $\frac{2}{3}$ (E × 2) Z.

FIG. 34.—*Homarus.* Retinulae partly isolated. Each retinula secretes a chitinous body which assumes a serrated appearance (*Rb*). This is the rhabdomere; when the bundle is brought together it assumes a spindle shape. The upper part of each retinula cell is free from pigment, and is connected with the pigmented portion by a long, slender neck. The nucleus of the retinula is situated at the non-pigmented, distal extremity. $\frac{2}{3}$ (D × 2) Z.

FIG. 35.—*Cambarus.* The retinula completely isolated. The rhabdomere shows a coarse wavy outline. ⅔ (D × 2) Z.

FIG. 36.—*Squilla,* partly diagramatic.

FIG. 37.—*Callinectes,* partly diagramatic.

FIG. 38.—*Serolis,* partly diagramatic.

The last three figures are introduced here so as to show the homology of the retinula cells in different groups of Arthropods.

All the specimens figured in this plate were treated and macerated in Haller's fluid.

PLATE XXXII.—*Limulus.*

FIG. 39. Young embryo of *Limulus.* *D. O.* "Dorsal organ"; * shows the position where the compound eye makes its appearance.

FIG. 40. Larval *Limulus* in the so-called "trilobite stage"; immediately after hatching. *D. O.* "Dorsal organ"; *E.* Compound eye; *Oc.* Ocelli.

FIG. 41. Young *Limulus* at the first stage in the acquisition of the external Limuloid characters. The whole body is transparent and the dendritic ramifications of the "liver" shining through the transparent tissues of the body. The "dorsal organ" has completely disappeared in this stage. *E.* the compound eye; and *Oc.* Ocelli.

FIG. 42. Transverse section of a young larva, showing the origin of the compound eye (*E*), the formation of the dorsal and ventral folds of invagination (*d.f* and *v.f*), and the "dorsal organ" *D. O.* ⅔ (A × 2) Z.

FIG. 43. Transverse section of the compound eye, showing the formation of the optic nerve fibres by the migration of the ectodermal cells. ⅔ (D × 4) Z.

FIG. 44. Outline of the thickening of the nerve plexus beneath the ommatidial layer. ⅔ (E × 2) Z.

FIG. 45. The same. ⅔ (D × 4) Z.

Both are drawn from the gold-chloride preparations.

FIG. 46. Ganglionic swelling found in the optic nerve fibres of the median eye. ⅔ (4 × D) Z.

PLATE XXXIII.—*Limulus.*

FIG. 45*a*. Longitudinal section of the eye, showing the formation of the optic nerve fibres (*Op. n*).

FIG. 46*a*. Oblique longitudinal section of the eye, showing the formation of the optic nerve fibres (*Op. n*).

FIGS. 47–56. Consecutive series of transverse sections of the compound eye of *Limulus* at the beginning of the lateral invaginations. Fig. 47, No. 1; Fig. 48, No. 3; (the section No. 2 is essentially the same as No. 3). Fig. 49, No. 4; Fig. 50, No. 5; Fig. 51, No. 6; Fig. 52, No. 7; Fig. 53, No. 8; Fig. 54, No. 9; Fig. 55, No. 10; Fig. 56, No. 11. The lateral invaginations become distinctly marked at Fig. 49, No. 4, and fuse in the median line at Fig. 54, No. 9, forming the median fold (*m.f*).

PLATE XXXIV.—*Limulus*.

FIGS. 57–64 show the condition of the lateral and median invaginations of the compound eye in the stage shown in Fig. 41, Pl. XXXII.

FIG. 57. In this the plane of section passes through the anterior part of the eye, and the dorsal invagination (*d.f*) of the compound eye is distinctly shown. The skin is thrown into folds; into the concave lumen of the fold a slightly thickened portion of the cuticle fits, forming the rudiment of the lens-cone (*C*); and the cells forming the walls of the invaginated fold form the beginning of the ommatidium.

FIG. 58 shows the widest portion of the eye in this stage; the beginning of the ventral invagination (*v.f*) is distinctly recognizable. The dorsal fold (*d.f*) is very large. Four distinct ommatidia are formed in this section, each depression being accompanied by a slight thickening of the corresponding portion of the cuticle (*C*). The basement membrane is very distinctly seen underneath the ocular area and beneath the general ectoderm cells which lie outside of the ocular area. The action of acid to which the sections were subjected in order to remove the pigment granules has interfered with the preservation of the ectodermal cells in those regions, although the thick, refractive basement membrane remains quite distinctly.

FIGS. 59, 60 and 61 show how the lateral invaginations are gradually approaching as we go towards the posterior part of the eye. In Fig. 62 the dorsal and ventral folds (*d.f* and *v.f*) meet in the median line, and in Fig. 63 they form a complete tube, the granular, non-pigmented layer coming to the outside and the pigmented cell layer (*Pg.c*) occupying the deeper part of the tube. The basement membrane (*B. M*) forms a complete capsule around the tube. The basement membrane which underlies the general ectoderm comes to

the outside of the median fold (*m.f*). Counting from the outside we meet with three consecutive strata of basement membrane in this region of the eye.

FIG. 64. In this we come nearly to the posterior extremity of the median fold (*m.f*). The median fold is further from the surface basement membrane than in Fig. 63.

PLATE XXXV.

FIGS. 65-69. Diagrams showing the probable evolution of the three-layered ommatidium from the single-layered surface depression in the skin, by the gradual subsidence of the neuro-epithelial elements, *Rt* and *G*, Fig. 65. In Fig. 66 the ommatidium of *Limulus* is represented, which is considered a step further advanced from the condition shown in Fig. 65. The distal end of the retinula (*Rt*) instead of being pointed toward the exterior as in Fig. 65, in *Limulus* it points towards the median axis of the ommatidium. The chitinous substance being still secreted on the outside, a distinct body of chitin beneath the lens-cone (*C*) is formed, the rhabdom (*Rb*). In Fig. 67 this deepening is supposed to have gone still further, resulting in the formation of another independent chitinous body, the crystalline cone (*C. c*). In Fig. 68 this deepening is considered to have advanced still further, the crystalline cone (*C. c*) being entirely separated from the corneal lens (*C*) by a distinct stratum of cell, the corneagen (*c. g*). In Fig. 69 an ommatidium with three strata of cells, each secreting chitinous substance on the part of their surface, is formed. These three strata of cells are known as the *corneagen* (*c. g*), the *vitrella* (*V*), and the *retinula* (*Rt*). Three chitinous bodies secreted by each group of cells above mentioned are the *cornea* (*C*), the *crystalline cone* (*c. c*), and the *rhabdomere* (*Rb*), respectively.

FIG. 70.—*Serolis.* Diagram of the ommatidium of *Serolis.* General arrangement of cells in this is not very different from that shown in Fig. 69. The place of ganglion cell in Fig. 69, *G*, is taken by a pair (of which only one is shown in the diagram) of transparent "hyaline cells" (*H*).

FIG. 71.—*Cambarus.* This is introduced in comparison with the hypothetical ommatidia.

FIG. 72.—*Callinectes.*

FIG. 73.—*Talorchestia.*

In the last three forms no element corresponding to the central

ganglion cell of *Limulus* nor to the "hyaline cell" of *Serolis* can be found. The sensory element of the ommatidium is represented by the retinulae (*Rt*) only.

FIG. 74.—*Limulus*. Diagram of the compound eye of *Limulus*, the black, heavy line representing the ectoderm and each depression in this layer corresponding to an ommatidium.

FIG. 75.—*Serolis*. In the same way as the above, the eye of *Serolis* may be represented by a series of folds.

FIG. 76.—*Notonecta*.

FIG. 77.—*Agrion* (Larva).

FIG. 78.—*Branchipus*.

FIG. 79.—*Cambarus*.

FIG. 80.—*Penaeus*.

FIG. 81.—*Lucifer*.

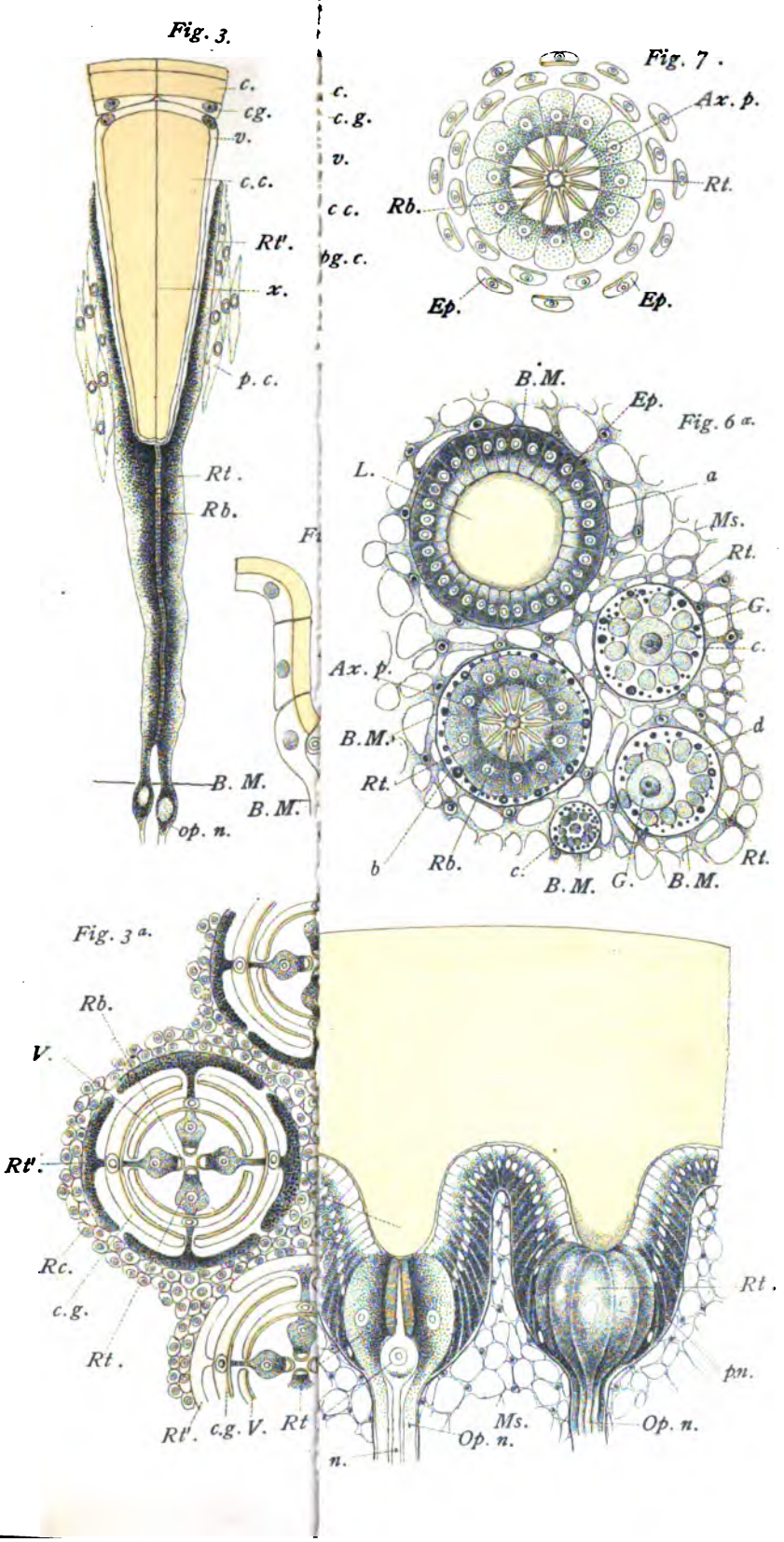


Fig. 3.
c.
cg.
v.
c.c.
Rt'.
x.
p.c.
Rt.
Rb.
B.M.
B.M.
op.n.
Fig. 7.
Ax.p.
Rt.
Rb.
Ep.
Ep.
c.
c.g.
v.
c c.
bg.c.
B.M.
Ep.
Fig. 6 a.
L.
a
Ms.
Rt.
G.
c.
Ax.p.
B.M.
d
Rt.
b
Rb.
c.
B.M.
G.
B.M.
Rt.
Fig. 3 a.
Rb.
V.
Rt'.
Rc.
c.g.
Rt.
Rt'.
c.g.
V.
Rt
Rt.
pn.
n.
Op.n.
Ms.
Op.n.
Op.n.

ON THE ANATOMY AND HISTOLOGY OF CYMBULIOPSIS CALCEOLA.

By J. I. PECK. With Plates XXXVI–XXXIX.

The specimens upon which the following researches were made were collected in the Gulf Stream, off Cape Charles, in October, 1888, by the Fish Commission schooner Grampus, and my thanks are due to the U. S. Fish Commissioner Prof. M. McDonald for affording me the opportunity to make the expedition, and also for putting the vessel and her crew at my service during the cruise.

It is the purpose of the present paper to give the anatomy of the pteropod *Cymbuliopsis calceola*, as obtained from a study of serial sections of the entire animal; also to indicate the histological elements of some of the principal tissues as they appear in their anatomical connection. The molluscs were taken at the surface of a moderately rough sea, at lat. 37° 11′ N., long. 74° 15′ W., in an early morning at the beginning of October, 1888. The specimens were killed in Perenyi's fluid and hardened in alcohol, by which process the tissues generally were well preserved, with some shrinking of the delicate connective tissue network, however, in the process of imbedding. A successful double-staining *in toto* was realized with dilute Kleinenberg's haematoxylin, followed by borax carmine.

The systematic position of the pteropod has been given by the new classification of the family Cymbuliidae as proposed by Pelseneer,[1] according to whom the new genus *Cymbuliopsis* is established, to include two species, which are distinct from Cymbulia on the one hand in having no "ventral lobe of the foot," nor "lash-like appendage" below the fin, also in having the flattened proboscis free from the upper surface of the base of the fin, and differing especially in the shape of the "slipper" or

[1] *Report on the Pteropoda collected by H. M. S. Challenger during the years* 1873–1876, Part LXV, p. 96.

"shell," which in Cymbulia is pointed at the aboral end and truncated at the anterior end, with long pointed tubercles along the margin. The genus also differs on the other hand from Gleba (Tiedmannia), inasmuch as the latter has a "shell" with a much more widely open cavity. The true shell, with operculum, of these forms, however, the homologue of the ordinary molluscan structure, only exists during larval stages,[1] having nothing in common with this secondary thickening of the mantle of the adult Cymbuliidae. I have therefore, referring to its present protective nature as a thick leathery armor into which the body of the animal slips as a helmet encloses the head, called this later structure in Cymbuliopsis—formed within the mantle—the *casque*, distinguishing it from the true shell of the mollusc, which was doubtless present in larval life.

The general form of the pteropod from a dorsal view is shown in Pl. XXXVI, Fig. 1, and that of the casque in Fig. 2, both drawn from a specimen in alcohol, in which the casque has slipped out of the mantle in which it was secreted, leaving the frayed portions of the exterior layer of the same still hanging from the body of the animal. The wide flattened proboscis, *p*, —the head region—is in contact with, yet free from, the upper surface of the fin. It is in fact in this case asymmetrical in form; a result perhaps of its shrinking in alcohol. Its edges are rolled outward to form a ciliated groove, which is, no doubt, sensory in function. Directly at the front part of the proboscis is the mouth, a large distensible opening regulated by loose lip-like folds. The position of the rudimentary tentacles—one upon either side of a median line over the oesophagus—is shown at *t*, and they are ordinarily discernible in surface views, although they are not shown in the figure. They are sometimes wholly absent, for in a very complete series of sections of one specimen they do not appear, while in another series they are well defined through at least ten sections, having a retractor muscle underneath and a nerve supply. But at best the tentacles are mere knob-like structures, with rudimentary sensory structure at their base, however, and they may be retracted to the general surface level of the proboscis. The oesophagus leads backward and

[1] See *Klassen und Ordnungen des Thierreichs*, Bronn und Keferstein—Malacozoa, pp. 630–632.

downward to the visceral mass or nucleus, seen through below and displaced to the left. All the tissues of the animal are exceedingly translucent, so that the course of the oesophagus may be followed from surface view. In perfect specimens the visceral nucleus is still enclosed in the pallial cavity by the internal layer of the mantle, represented in the figure by a circle drawn a little way exterior to the visceral nucleus, but this latter is generally more or less torn in the loss of the casque at the capture of the animal. This dark-brown rounded "nucleus" contains the digestive and reproductive organs, and on its dorsal surface the heart and nephridium are laid. The primitive molluscan foot cannot be recognized in the adult animal, while its successor, the fin, has attained very large relative proportions, and is moved by muscles which are laid in regular intercrossing bands a little beneath the epithelial surface of either side, both dorsal and ventral alike; the thickness of the fin being made by the branching network of connective tissue cells so characteristic of molluscan histology. The structure and disposition of these muscle bands has been recently described for other pteropods by Paneth,[1] and in comparison with the heteropods by Kalide,[2] but without especial reference to Cymbuliopsis. In the latter genus there are three definite layers of bands controlling the fin, Fig. 1, 1, 2 and 3. Of these the most exterior bands are (1) the narrowest, and are distributed nearly parallel to each other over the whole surface of the fin in an antero-posterior direction. These lie directly upon and across the next layer beneath them (2), which is made up of thicker and much broader bands running across the fin from one side to the other in arcs of circles. And finally the innermost bundles (3) are distributed radially from beneath the proboscis in such a way that the two lateral portions of the fin are supplied by them, a few bands a little to the right and left of the median line in front of the proboscis being omitted. All the muscle bands as they near the edge of the fin become small and branch out to anastomose with each other. The edge

[1] *Beiträge zur Histologie der Pteropoden und Heteropoden,* Archiv für mik. Anat., Bd. XXIV, pp. 235, 262.

[2] *Beitrag zur Kentniss der Muskulatur der Heteropoden und Pteropoden,* Zeit. für Wiss. Zoöl., Bd. XLVI.

of the fin also contains branched connective tissue cells and processes passing from a central cell nucleus to both surfaces. In the general interior of the fin, of course, is the network of connective tissue cell processes, and the branching nerve trunks and blood lacunae. The mollusc also possesses six longitudinally laid muscle bands on the dorsal surface of the proboscis. The relations of the parts in place from a ventral view are shown in Fig. 3. The visceral nucleus, from this view quite surrounded by the pallial cavity *k* (bounded by the circumscribing line in the diagram), shows at its aboral end, at *z*, a peculiar darkly pigmented ring, which is superficially placed and presents differences in size in different specimens; when the circumference of the ring is large the wall is low, and when contracted it is more prominent and stands out more upon the visceral mass. This limiting wall is made up of the characteristic branched cells, with single and branched muscle fibres (Fig. 27, *m*) cut at different angles, and it carries on the inside a layer of columnar cells which are much elongated in the bend. Pelseneer[1] has also mentioned this structure, and as being like a sucker in form. In Fig. 27, Pl. XXXIX, is represented a vertical section through one side of the ring in a specimen where it is much contracted. The pigment is distributed in the protoplasm of the columnar cells, whose nuclei are also a dark brown.

The pallial cavity of Cymbuliopsis is on the ventral side, and is made by the large fold of the mantle which extends from the dorsal part of the animal around beneath the fin, thickening between its two epithelial layers into the hyaline casque. (This latter appears in sections to be deposited in irregular layers, as it stains with difficulty except in streaks, and shows granules which are doubtless deposits of inorganic salts; it does not contain cells.) Part of the inner layer of the mantle is specialized into the "pallial gland," or "shield" of thecosomatous pteropods. The position of this—seen through from the exterior—is shown in Fig. 3, *p.g.* It is almost symmetrical in this genus, being twisted somewhat to the right, but the asymmetry is not marked, and it is crossed by two transverse bands—an anterior small one and a broader posterior one—somewhat faintly shown in the figure, also one passing along the edges of the structure a part

[1] *Challenger Reports*, Part LXVI, p. 27.

of the way. Sections through this "shield" show that the cells which compose the different areas are quite different in their appearance. Thus in Pl. XXXIX, Fig. 26 shows in section the long club-shaped cells *t*, which cover the general surface of the pallial gland on the side toward the cavity, and stain very deeply, while the transverse bands are made up of the cells *v*, which stain more lightly, and are regularly arranged so as to give the surface an undulatory contour. The outer portion of these closely packed cells *v* is clear, and between the larger cells, near the surface, are found certain smaller nuclei *n*. The transition between the two areas is very abrupt, and the function of the two, whatever it may be, is doubtless as distinct. Behind the pallial gland, which thus forms a widely open pocket, the general pallial cavity is much narrowed down, opening into that part which surrounds the visceral mass by a transverse crescentic slit (below *k*, Fig. 3;—at *y*, Fig. 4). This posterior part of the cavity contains the visceral nucleus suspended in it, but it is asymmetrical in extent on account of the presence of the large nephridium, which extends farther down upon the left side, so that the pallial cavity extends farther dorsalwards on the right side than on the left, while behind it extends quite up on to the dorsal side as far as the large nephridium. Thus in Fig. 4 are shown the relations of the parts in diagram from the right side; *m* the mouth at the proboscis, *t* the rudimentary tentacles, *n* the central nerve ganglia, *k* the casque contained in the mantle, *j* the nephridium with the heart in front of it, *y* the opening from the larger exterior part of the pallial cavity with its pallial gland *p. g.* into the interior part of the same, where open the anus and the genital ducts at *w*. The external features of the mollusc, the extent of the mantle, pallial cavity and shield, the position of the visceral organs, the fin and its musculature have thus been indicated in Pl. XXXVI, Figs. 1–4; the structure of the shield and dark ring or sucker in Pl. XXXIX, Figs. 26 and 27.

In order to show the arrangement of the organs of the visceral mass I have figured on Pl. XXXVII (Figs. 8, 9 and 10) three representative cross-sections at different points through the body of the animal. The outlines of the visceral organs were made with the camera lucida, while the outer epithelium (*k*) of the

mantle and the inner layer (*w*) of the same, and the intervening thickening—the casque—are represented diagramatically in place. Fig. 8 is the most posterior section, the pallial cavity quite surrounds the visceral mass, the nephridium *n* is just cut on the dorsal side as a double cavity at this point. The intestine of Cymbuliopsis, after leaving the stomach, makes a single loop around the visceral nucleus (Fig. 4, *i*) under the genital gland, and opens a little to the *right* of the median line; and here, in Fig. 8, is cut so that the coil is just disappearing at *i* on the left, while the part of the intestine leading directly to the anal opening is cut at *i* on the right. The beginning of the enlargement of the stomach *s* is on the dorsal side, and opens widely into the liver *l*, the opening being to the right of a median line. The liver *l* is quite internal then, and the gland is made up of loose lobules. The genital gland *h* is most exterior, and envelops the liver and intestine. In Fig. 9, farther forward, about the middle of the visceral nucleus, the nephridium is cut in two parts, right and left, *r. n* and *l. n*, while between them appears an irregular part of the pericardial cavity *p. c*, in which the ventricle would appear more anteriorly. This section passes about through the centre of the stomach *s*, with the five thickened plates upon its walls. A very large blood sinus *ao* passes below the stomach into the tissues of the liver *l*, which, with the genital gland *h*, occupies the same relative position as in the other figure. At *a* is seen the terminal portion of the anal opening into the pallial cavity, a little to the right of the median line; although in surface views it is seen to be directed toward the left, yet it does not reach the median vertical line. The pallial cavity itself in this, as in the following sections, is seen to extend farther dorsalwards on the right side than on the left. Fig. 10 is taken far enough forward to pass through the ventricle *v*, just before its union with the posterior blood-vessel *ao*; the nephridium, *r. n* and *l. n*, has almost disappeared on either side, while the pericardial cavity *pc* is becoming more apparent, and would steadily increase in the more anterior sections of the series until it is limited by the general connective-tissue filled space of the body cavity. The stomach *s* is cut through its anterior end, where only two of the thickened plates are developed; the liver has almost disappeared, and the genital gland *h*

is divided into two lateral portions by the ventral position of the accessory glands and the genital ducts, to be described hereafter. In following serial sections farther forward, the liver and the genitalia run out, the stomach runs into the oesophagus, the heart gives off the large aorta, the mantle shows the specialized pallial gland, the nerve ganglia appear around the oesophagus, and the general tissues of the fin and proboscis appear.

The Digestive Organs.

The general position of these has already been described; beginning with a large, loose mouth at the proboscis, from which the long oesophagus leads backward into the stomach, the thickened walls of which bear the triturating plates. At the extreme posterior end of the stomach, at the beginning of the intestine occurs the wide asymmetrical opening into the "liver" (Fig. 8), while the intestine itself takes one bend downward and around over on to the right side and beneath, where it opens nearly upon the median line, although plainly to the right of it, which is contrary to the position described for the other Cymbuliidae. Its latter portion is directed toward the left, as it opens into the pallial cavity. Cymbuliopsis has no buccal armature nor salivary glands; the oesophagus is lined with ciliated epithelium which is raised into secreting folds, Pl. XXXVII, Fig. 11, the whole being contained by longitudinal and circular muscles *m*. In the stomach these folds become brown pigmented villi (seen in diagram around the inside of the cavity *s*, in Figs. 8 and 10) without cilia, the cells of which are thickly distended with drops of secretion, their outer boundaries being blended together, Fig. 12, and the musculature *m* becoming thicker. Of the five thickenings of the stomach walls usual in the thecosomatous pteropods, the fifth, or smallest plate, is in this genus situated on the dorsal side between the pair of larger dorsal plates. All are included in the section from which Fig. 9, Pl. XXXVII, is taken. Besides these five constant ones there are several other irregular spur-like plates of the same structure, placed sometimes in the spaces between the larger plates, or in either end of the stomach. The structure of these thickenings in the walls of the stomach is shown in Fig. 13, Plate XXXVIII, the section being figured from near the edge of one of the ventral plates, at

about the place indicated by x in *s* of Fig. 9. Most exterior are the large nucleated fibres of the circular muscle coat, which control the movements of the stomach; within these is the thickening which carries the large tooth-like plate. This thickening is merely an accumulation of the "gallertgewebe"[1] of the mollusc, as is shown by the branched cells, laid in a matrix which is clear; the network of cell processes is more numerous than is indicated in the drawing (those between the cubical epithelium and the muscle coat), the nuclei of which exist often in pairs and sometimes even in threes, as if growth were taking place. Placed upon this connective tissue thickening of the wall is a single very regular layer of cubical epithelium, which secretes toward the lumen of the stomach the projecting pyramidal "tooth." These latter are of the same general structure as the casque secreted by the mantle, being hyaline, staining with difficulty in streaks, and containing a granular deposit. In Fig. 13 the circular muscles, the connective tissue and the secretory epithelium are tinged with blue, the secretion of the "tooth" at that point being represented in yellow. These plates have been regarded as triturating structures in the pteropods, and the "stomach" has been called a "crop," with reason, doubtless, in this case where there is no radula nor jaws at the mouth. But the lack of this structure (radula) does not prevent the animal from possessing predatory habits, for in one of these specimens an adult heteropod, Firoloidea, was found, having been taken as food; but the stomach is usually full of diatoms, foramenifera, etc., of many kinds.

The digestive gland, the "liver" of the mollusc, constitutes a large part of the visceral nucleus. Its wide asymmetrical opening into the intestine has already been described with Fig. 8, while the character of the cells that compose it is illustrated in Pl. XXXIX, Fig. 20. These are the kind described as "körnerzellen" by Frenzel,[2] and in Cymbuliopsis they are characterized by large greenish-brown masses in various stages of perfection. In some cells there are solid, irregularly spherical, greenish masses; in other cells there is only an irregular central mass in a vacuole-

[1] See also Paneth, *l. c.* p. 255.

[2] *Ueber die Mitteldarmdrüse der Mollusken*, Archiv für mik. Anat., Bd. XXV, p. 52.

like portion of the cell, Fig. 20, *j*. They are all, doubtless, steps in the same process, and sometimes the whole cell is tinged with green without its being aggregated. I have also found these masses outside the cells in the lumen of the alveoli. There are also clumps of very large opaque granules in the peripheral portion of very many of the cells. There are, moreover, rods of cells at various points in the periphery of the liver, the cells of which are smaller than, and very different from the glandular portion of the gland, and in the centres of these rods there are small transparent masses of crystals. I could not make out the exact form of the individual crystals, and appearances favor the assumption that they are derived from the salts in solution in the blood and tissues of the animal, and that the rods may be in reality short ducts with very thick walls, but I cannot describe even their general direction. The blood permeates the "liver" very plentifully, as may be inferred from the large blood-vessel which supplies it, Fig. 9, *ao*, and which is distributed into the blood spaces in the walls of the basement membrane between the alveoli.

In the centre of this digestive gland are large irregular spaces (Pl. XXXVII, Figs. 8–10, in the brownish lobulated mass *l*), into which food products from the stomach may pass in the process of digestion. There are also tracts of ciliated liver cells, situated at various points along the central spaces; they line the passage from the intestine out into the liver, and serve doubtless for transporting food particles, etc., whenever they occur. Thus in Fig. 8 at *y* is seen the relative position of one of the ciliated pads, while the cells represented in Fig. 20 at *i* are taken from a part of the gland which projects into the central space; the cells at *i* are specialized into a ciliated tract, unlike the ordinary cells of an adjoining fold of the liver at *j*. The "liver" of Cymbuliopsis, therefore, is something more than a mere secretory appendage, and comes into direct contact with the food circulated within it. The intestine is quite thin-walled, and lined with simple short columnar ciliated cells, thrown into a few villi in the anterior portion.

The Central Nervous System.

The nerve ganglia of Cymbuliopsis conform to the type of the Cymbuliidae in general, as it has been described in detail for

Cymbulia,[1] with some additions to the descriptions previously given. The general position of the ganglia in the body is shown in Fig. 4, *n*. They are very closely fused, as is shown from ventral view in Fig. 5, which was reconstructed from camera drawings of serial sections. The cerebral ganglia *r. c* and *l. c* (right and left cerebral) lie at the sides of the oesophagus, and are connected to each other around it by the large cerebral commissure *cc*. On the extreme dorsal part of this commissure, *i. e.* in the true morphological position of the cerebral ganglia, there are sometimes a few ganglion cells remaining, but without giving rise to nerve trunks. From the cerebral ganglia two pairs of nerves are given off anteriorly into the proboscis, and one short pair to the otocysts below them, although this pair is much hidden from surface views. The more dorsal pair of cerebral nerves c_1 go to the tentacles and supply the ganglionic swelling at their base. Their other pair c_2 go to the proboscis, supplying the ciliated lips.

The pedal ganglia—connected in Cymbuliopsis, as in Halopsyche and the gymnosomatous pteropods, by a second delicate commissure *s*—give off three pairs of nerve trunks p_1–p_3, which proceed to the fin; p_3 being smaller and supplying the proboscis. There is also a very delicate nerve, *m*, given off from the posterior ventral face of the pedal ganglion, and sinking almost directly to the ventral surface of the base of the fin.

The nerves which pass backward into the body are derived from the visceral and buccal ganglia. Of the former there are three ganglia, giving off the four nerves v_1–v_4, of which v_1 on the right side soon branches, sending one branch back into the mantle above, while the larger branch sinks below and supplies the mantle beneath the fin, and on this side carries the osphradium; v_4 is distributed in a similar way upon the left side of the animal, except that no sense organ is developed on the lower branch; v_2 comes soon to lie close to the oesophagus with the nerves from the buccal ganglia, with which it extends into the visceral nucleus, being laid in the right dorsal region just beneath the nephridium and circulatory organs. The large left branch v_3 bends below, and I have traced it far out into the mantle, along

[1] *Recherches sur le system nerveux des Pteropodes*, par Paul Pelseneer, Archives de Biologie, Tome VII, fasicule 1, p. 115.

the edge and beneath the pallial gland. The fused buccal ganglia are situated in front of the pedal, close to the oesophagus. They give off anteriorly a pair of slender nerves b_2 to the oesophagus, and posteriorly a similar pair, b_1, that run close to the wall of the stomach.

On Pl. XXXVIII, Figs. 14–17, are figured four representative cross-sections through the central ganglia in place. Fig. 14 is the most posterior section, and includes the ring of cerebral and visceral ganglia, the former of which are represented as giving off the cerebral commissure *cc*, above the oesophagus, with the dotted lines; which commissure, however, has been cut in the preceding sections of the series. The cerebral *c*, pass freely into the visceral ganglia *r. v*, *m. v*, *l. v*, and they into each other. Among the nerve cells, located peripherally in the ganglia, there is in the median visceral ganglion *m. v*, situated upon the left side at *k*, a very large bipolar cell, whose processes may be traced to either side into the other ganglia. It is a constant feature of all the specimens examined (four) in the sections through the central ganglia, and in one instance had a diameter of 120μ, while the greatest diameter of the whole ganglion was 225μ. This cell, then, may be taken as presenting the qualities of all the cells of its kind in the periphery of the ganglia. In Fig. 18 I have endeavored to indicate the disposition of the chromatin elements in the large irregular nucleus, which has perhaps taken its present form on account of the shrinkage of the body of the cell, and this is the case with many of the larger cells in section. One of the two or three protoplasmic processes *p* of this large cell is passed through in this section. Fig. 15 is taken ten sections forward. Here the otocysts *o* are cut in section, and the auditory nerve *n* on the right side, the fibres leading upward to a bunch of small cells of the cerebral ganglion; the plane of section being a little oblique, the left auditory nerve was just previously cut in the series. The otocysts in these specimens of Cymbuliopsis contained no traces of otoliths, nor was the internal epithelium at all specialized into ciliated sensory cells, but this may have been caused by the action of acid in the reagents. Although the otocysts lie upon the pedal ganglia, yet they are bound to the visceral ganglion *m. v* by two strands of connective tissue from their investment. In this section also is shown the

origin of the small nerve m, whose fibres are given off from the large cells of the internal posterior face of the pedal ganglion; also the origin of the cerebral nerve c_1 from the dorsal side of the cerebral ganglion $l.\ c$—not apparent in this plane through $r.\ c.$ The buccal ganglia b in the median line just below the oesophagus still show a partial segmentation.

Fig. 16 is four sections in front of Fig. 15, showing, upon the left side, part of the commissure cb which exists between the cerebral ganglia c and the buccal ganglia, and in the same section the point at which a similar connection is to be established with the buccal ganglia from the right side opposite; and also the origin of the pair of buccal nerves b_2 which pass forward from the buccal ganglia, close to the oesophagus; also a part of the elongated origin of the pair c_1. The whole of the central nervous system, including the large nerve trunks, is sheathed in a thin connective tissue envelope, the small cell-nuclei of which are very numerous. Some of this investment cut tangently from the surface of the pedal ganglia is shown in Fig. 16, g; it is likewise seen in all the sections as a limiting membrane of the ganglia.

The pedal ganglia alone exist in the section from which Fig. 17 is taken, the fibres pass from one freely into the other and out into the large trunks of the pedal nerves, the origin of p_1 and p_2 being shown upon the left. The nerves from the cerebral and buccal ganglia are seen above in section, as they pass forward into the proboscis.

The osphradium—Spengel's olfactory organ—of Cymbuliopsis is quite large and long, extending on the right side of the mantle as it narrows to form the opening into the inner part of the pallial cavity. It is composed of ciliated epithelium with ganglion cells beneath, from which fibres of the nerve pass. Fig. 19 shows the arrangement of the epithelium in two patches, a condition which obtains for some distance. There is also a ciliated pad of cells, Fig. 19, a, within the pallial cavity upon the visceral mass, of which mention will be made hereafter; but other than these and the tentacles, the edge of the fin and proboscis also serve as sensory organs, partly as organs of special sense, no doubt.

The Reproductive Organs.

The position of the generative gland has already been shown in Figs. 8–10, *h*. It is distributed over the visceral nucleus as a uniform external layer; is divided up into large follicles by a very thin limiting membrane, brown pigmented in general, of which the internal layer—toward the liver—is more or less free from the general tissues of the gland, so as to form loose spaces in which the genital products may be carried toward the efferent duct and hence to the exterior.

In the specimens of this pteropod examined, the hermaphroditic gland was in the state of female activity; that is to say, the male elements had been matured and shed, while the following development of ova was in process. And so there were no primitive germ cells developing into spermatozoa on the internal side of the gland, while the outer portion of it is filled with ova in all stages of maturity. The changes which take place in the growth of the ovum from the primitive germ cell are shown in the reproductive follicles figured in Pl. XXXIX, Figs. 22 and 23. The germinal cells destined to become ova enlarge—some more rapidly than others, as is shown in the follicle *a*, Fig. 22, and these latter are to complete their development while the other cells contribute only indirectly to the production of ripe ova by furnishing food material for the more vigorous neighboring cells. These latter cells, therefore, increase in size at the expense of the others, and so gradually take on the characters of the ripe ovum in their growth. Thus in the follicle *b* are seen two young ova surrounded by a layer of the other germinal or follicular cells; the nucleus or germinal vesicle is very large and clear, except for chromatin elements scattered about, and in the ovum upon the left of the follicle *b* the nucleolus, or germinal spot, is cut in section, the body of the ovum being granular protoplasmic material. In the follicle *c* a still older stage is shown, the ovum being farther advanced, and evidently using the large irregular cells which it adjoins, while the rest of the follicle is occupied by another ovum which only partially appears in this particular section.

In the further growth of the ovum, food-material is deposited in the protoplasm in the form of large yolk spheres, which have

characteristic forms and markings for different thecosomatous pteropods.[1] The markings upon the yolk spheres of Cymbuliopsis give them the appearance of minute dense cells with a nucleus, but thickened on one border. In follicle *d*, Fig. 23, the protoplasm of the largest ovum is being furnished upon its upper border with the first of these yolk masses. From the beginning these latter are perfectly distinguishable by the manner in which they take the staining used, *i. e.* they remain untouched by the haematoxylin and only take the bright carmine color. Finally, in follicle *e* the ovum has acquired all the characters of ripeness, and in this particular section appears the large nucleus, with the nucleolus *k*, and a great abundance of nutritive material stored up as yolk-spheres *y*. After completing its growth to this stage it is loosened from the follicle and is ready for fertilization.

The genital ducts and the accessory glands are diagramatically represented in Pl. XXXVI, Fig. 6. The common genital duct *d* is quite short, leading from behind down and around to open upward into the uterine portion of the duct *u*, which itself leads backward on one side, into which the seminal receptacle *r. s.* opens, and downward to the exterior on the other side at *h*. The two accessory glands—displaced a little downwards and to the left in the diagram—open very widely into the terminal portion of the duct. The albumeniparous gland is the smaller and exterior, the muciparous is the larger and interior, both being thrown into loose folds. The relative position of these organs in the visceral nucleus is shown in Fig. 4 at *w*, also in the section Fig. 10, the duct and opening being on the right of a median line, the accessory glands occupying nearly a median position in the body. In the specimen here figured the seminal receptacle *r. s.* is filled with spermatozoa, and a portion of the duct which connects it with the duct to the exterior (*e*) is cut at *f* (although it of course soon runs out in the following sections). The walls of this uterine portion are much folded, brown pigmented and ciliated, with a few spermatozoa visible in its upper portion. The genital duct d—d_1, leading from the hermaphrodite gland to the uterus, is cut twice in this section; farther on in the series they unite with each other. Although in this mollusc

[1] See Fol, *Sur le developpement des Pteropodes*, Archives de zoologie exp. et gen., Tome IV, p. 106.

there is but one genital duct common to both sexual elements, yet this is specialized into two portions, as seen in Fig. 24, one portion of a section across the duct being lined with larger ciliated cells than the other portion; a condition which may be analogous to that of those gasteropods where one part of the "oviduct" is specialized into the seminal groove. The duct becomes larger at its lower portion, as seen in Fig. 25, where a few yolk spheres *f*, with the round marking, are included from a passing egg.

The folds of the muciparous gland *m* are much longer than those of the albumeniparous gland *a*. The structure of these is further represented in Pl. XXXIX, Fig. 21, which shows that the cells of the one gland—the muciparous—are larger and clearer than those of the other, both kinds being very regularly arranged upon a thin basement membrane with its occasional nuclei. In addition to the regular basal row of nuclei of the gland cells there is another incomplete row of smaller nuclei *n*, near the lumen of the gland fold, which appear to belong to small cells wedged in between the upper portions of the large gland cells; in some parts of the glands these nuclei are much more numerous than in the figure. It was also shown that analogous small nuclei existed in the pallial gland cells. I do not know their meaning. Cilia are also present in some parts at least of the glands.

Not only is the generative gland, in all these specimens, in a state of female activity, but I have been quite unable to find any penis to indicate that they ever function as males, or at least so as to effect a copulation among themselves. In the case here described and figured the seminal receptacle is filled with spermatozoa (in other instances it was empty) and ova are passing through the genital duct, but it is not probable that both the sexual elements were derived from the same individual gland, and a penis therefore probably exists in some other individuals functioning as males. This organ is described for the other Cymbuliidae as a protrusible tube lying upon the dorsal side of the animal, with its exterior opening a greater or less distance behind the tentacles; and connected with the external opening of the genital glands by a ciliated groove for the conveyance of the male element. There is indeed in Cymbuliopsis a narrow band of ciliated cells (Fig. 19, *a*) leading away from the external

opening of the genital organs, but it becomes less and less conspicuous in its anterior course until it is lost near the opening into the inner pallial cavity; but it does not lead into any groove or cavity, nor is there any indication of its relation to any penis, nor does the latter appear at all in the serial sections. This element of the structure of a functionally male pteropod is therefore only rudimentary, and it is possible that the germ cells never produce functional spermatozoa, but always ova, thus effecting a true separation of the sexes in many cases, at least, in this genus. Or perhaps the lack of a penis is only another one of the peculiar modifications of the mollusc, such as the want of a buccal armature, or the occasional lack of tentacles.

The Nephridium and Heart.

The general position of these organs in the body has already been indicated in Fig. 4 at *j*; their relations to each other are shown in diagram in Fig. 7, seen from the dorsal surface, the anterior direction being toward the bottom of the page. The nephridium of Cymbuliopsis is a single large loose sac lying above the visceral nucleus, across the pallial cavity, and bent in a horseshoe shape around the heart and pericardium which lie in front of it. Its position is quite superficial, and parts of its cavity are separated from the visceral mass. It is also asymmetrically disposed (the right lobe *r. n* being smaller than the left *l. n*); extending farther down upon the left side of the body than upon the right, Fig. 9 *r. n*, *l. n*, and extending out above the pallial cavity upon the right side, while the pallial cavity lies above it upon the left. Posteriorly its cavity extends out above and also beneath the pallial cavity, so it is cut twice in the section Fig. 8, *n*. It communicates upon the right side with the pallial cavity by a large ciliated opening *o* surrounded by a muscle, but this is the only opening that appears in the sections; and a second communication—with the pericardium as is the rule in the Mollusca—has doubtless been lost. It is also stated by Bronn and Keferstein that this opening into the pericardium has never been observed in Tiedmannia, so that in these points also these two genera seem to be similar.

[1] Klassen und Ordnungen des Thierreichs—Malacozoa, p. 609.

In front of and above the cavity of the nephridium, lying between its two large horns, is the pericardium, within which is the large ventricle. The pericardium begins as a recess lying above the nephridium, Fig. 9, *pc*, and broadens out anteriorly. It is shut off from the other parts of the body cavity by a layer of the connective tissue network. I have indicated in diagram, Fig. 7, the position of the two large blood-vessels connecting with the ventricle, and have called the anterior one, with a spacious sinus just in front of the ventricle, the auricle *t*; the other thicker-walled vessel *ao* passing backward over the visceral nucleus is evidently an aorta leading from the ventricle to the liver (in section Fig. 9, *ao*) and the other organs (Fig. 8, *ao*). The vessel *t* passes far forward into the fin, becoming rather smaller in its course and opening out into a very loose network of large lacunae just anterior to the central nerve ganglia; in none of all the tissues indeed do the characters of the molluscan branched connective tissue cells appear so clearly in sections as in this region just anterior to the pedal ganglia. There are large loose spaces in the body cavity in front of and beneath the ventricle, and I had expected to find in one of these the auricle of the circulatory system as it has been described in pteropod anatomy—*i. e.* as being an incompletely closed sinus into which the blood from the tissues is collected and from which it passes into the ventricle; but the sections of the material at hand were insufficient to warrant such conclusions. Furthermore, these spaces before mentioned contain certain lateral and dorsal areas of closely aggregated cells that do not differ much, except in greater size and abundance, from the other cells about the visceral organs, but which present a peculiar appearance in section through their mass. Their significance I cannot determine by a study of this form alone.

The lack of material is my only plea for the insufficient way in which the circulatory system of Cymbuliopsis has been followed up, and for the incomplete nature of the sexual differences. But in other respects it is hoped that the general pteropod structures have been indicated; and this genus seems to be generally typical of the whole family.

Explanation of Plates.

Fig. 1. Surface view of Cymbuliopsis from dorsal side; *p* proboscis, *t* tentacles, 1—3 muscle bands.

Fig. 2. Casque, from dorsal position when in place.

Fig. 3. Diagram of the parts in place, from ventral view, twice the natural size; *p. g* pallial gland with the bands across it, *k* the pallial cavity entered by a narrowed opening behind the pallial gland from this view, *z* the dark aboral ring.

Fig. 4. Diagram of the organs in place in optical section from the right side; *m* mouth, *t* tentacles, *n* central nerve ganglia, *j* nephridium with heart in front of it, *k* casque within the layers of the mantle, *i* intestine, *w* accessory genital glands, *y* opening from large to the interior pallial cavity, *p. g* pallial gland.

Fig. 5. Central nervous system: p_1—p_3 pedal nerves, c_1—c_2 cerebral nerves, b_1—b_2 buccal nerves, v_1—v_4 visceral nerves, *m* small posterior pedal nerve, *s* small pedal commissure, *o* otocysts, *r. c*, *l. c* right and left cerebral ganglia, *cc* cerebral commissure.

Fig. 6. Diagram of genital duct and accessory glands, *d* genital duct, *r. s* seminal receptacle, *u* uterine portion of the duct, *h* external opening.

Fig. 7. Diagram of nephridium and heart from dorsal side; *t* auricle, *v* ventricle, *pc* pericardium, *ao* aorta, *o* opening into pallial cavity, *r. n*, *l. n* right and left lobes of nephridium.

Fig. 8. Section across the more posterior part of the visceral nucleus; mantle and pallial cavity put in in diagram; *k* external layer of mantle, *w* internal layer of same, lining the pallial cavity, *s* stomach, *i* intestine, *h* hermaphroditic gland, *ao* aorta, *n* nephridium cut in two parts at this point, *l* liver—the letter itself being placed in one of the spaces of the same; *y* one of the ciliated projections of liver cells.

Fig. 9. Section through about the middle of the visceral nucleus; *r. n*, *l. n* right and left portions of the nephridium, *pc* pericardium, *a* anus, other letters as in the previous figure.

Fig. 10. Section across a more anterior portion of the visceral nucleus; *v* ventricle, *m* folds of the muciparous gland, *a* of the albumeniparous gland, dd_1 genital duct cut in two places, *r. s* seminal receptacle, *f* fragment of uterus, *e* leading to the external opening, other letters as before.

Fig. 11. Folds of lining of oesophagus; *m* muscle fibres, circular and longitudinal.

Fig. 12. Villi of beginning of stomach; *m* muscles.

FIG. 13. Portion of a thickened part of stomach wall, with secretion.

FIG. 14. Section through posterior part of central ganglia; *c* cerebral ganglia, *r. v*, *l. v* right and left visceral, *m. v* median visceral with the large ganglion cell *k*, b_1 buccal nerves, *cc* cerebral commissure, *oe* oesophagus in outline.

FIG. 15. Section through about the middle of the central ganglia; *r. c*, *l. c* right and left cerebral ganglia, c_1 origin of the pair of nerves c_1, *b* buccal ganglia, *l. p* left pedal ganglion, *m. v* median visceral, *o* otocysts, *n* auditory nerve.

FIG. 16. Section taken four sections anterior to that of the last figure; *c* cerebral ganglia, *r. p*, *l. p* right and left pedal, c_1 origin of the nerves c_1, b_2 origin of the nerves b_2 from buccal ganglia, *cb* cerebrobuccal connective, *g* connective tissue sheath of ganglia.

FIG. 17. Section through the pedal ganglia (*r. p*, *l. p*) with origin of large nerves cut upon the left side, c_1c_2 cerebral nerves, b_2 buccal nerves.

FIG. 18. Large ganglion cell; *p* protoplasmic process.

FIG. 19. The osphradium with nerve.

FIG. 19*a*. Ciliated band on the visceral nucleus.

FIG. 20. "Liver" cells; *i* with specialized ciliated cells, *j* ordinary secreting cells, with various cell contents.

FIG. 21. Section across the folds of the accessory genital glands; *m* muciparous, *a* albumeniparous gland, *n* the smaller exterior nuclei.

FIG. 22. Growth of ovum; *a*, *b*, *c* successive stages in a series of follicles.

FIG. 23. *d*, *e* older stages in the same series, *y* yolk spheres, *k* nucleolus.

FIG. 24. Section across the genital duct; large muciparous gland cells in outline.

FIG. 25. Through lower portion of the genital duct; *f* yolk spheres of a passing egg.

FIG. 26. Section in pallial gland; *t* the cells of the dark areas, *v* those of the lighter transverse bands, *n* small exterior nuclei.

FIG. 27. Vertical section through one rim of the aboral ring, *m* muscle fibres.

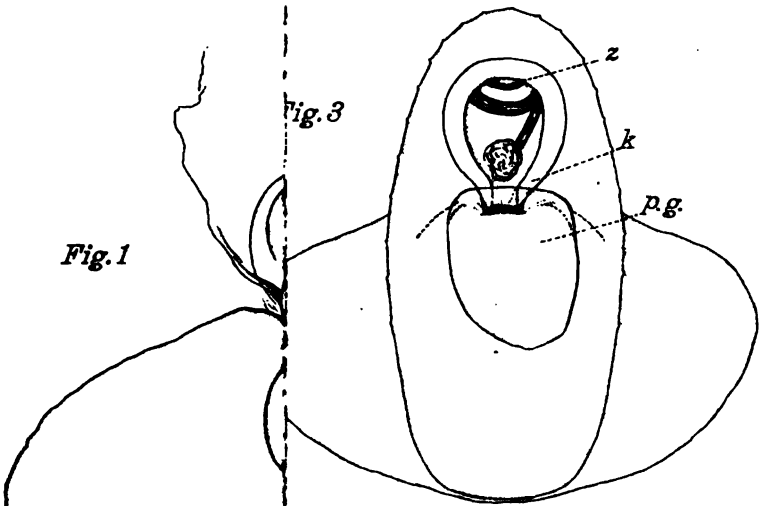
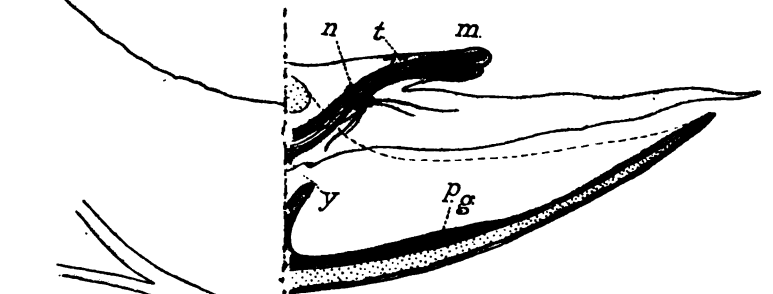
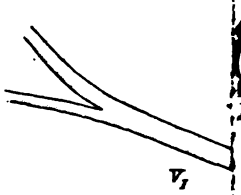
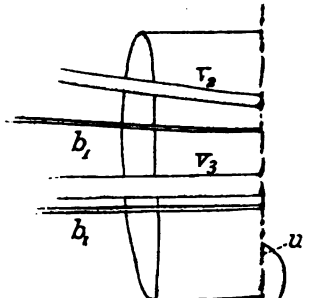


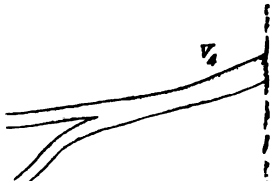
Fig. 3

Fig. 1









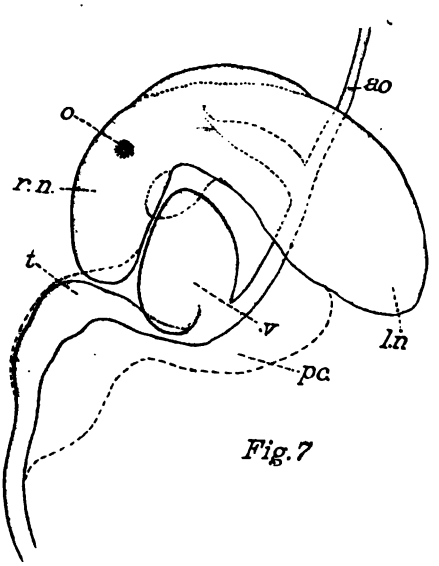


Fig. 7

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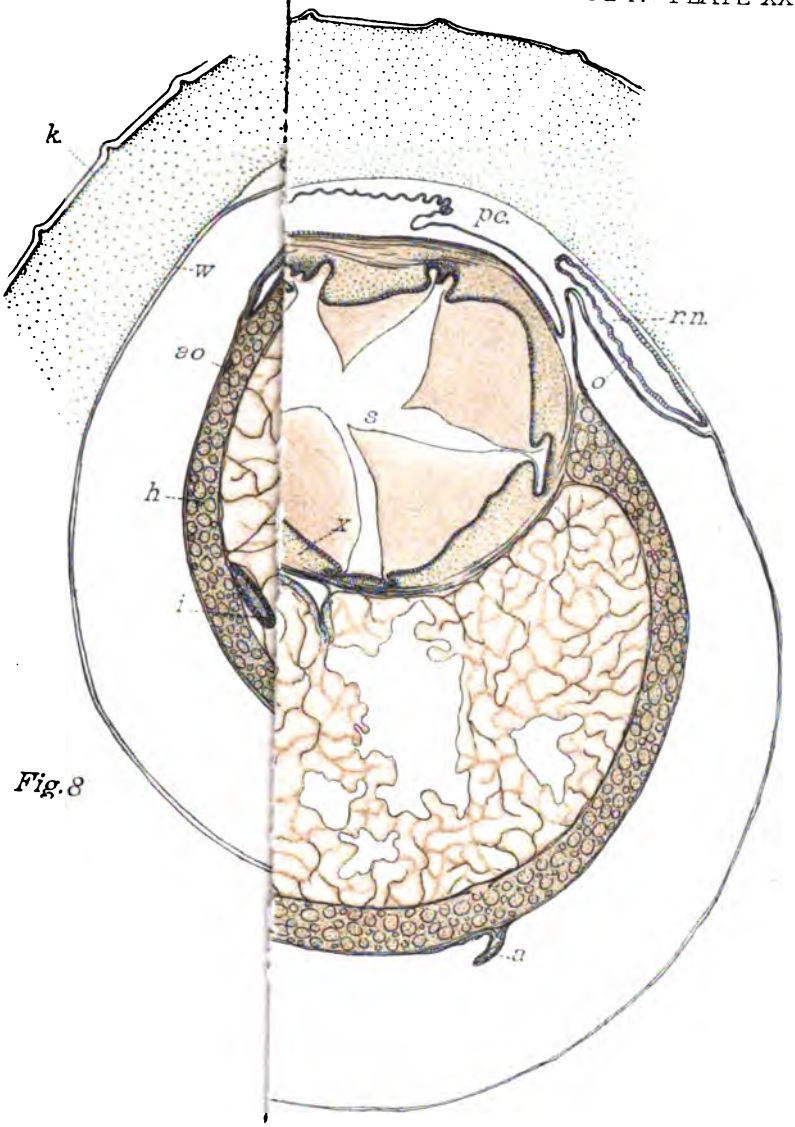


Fig. 8

Fig. 11

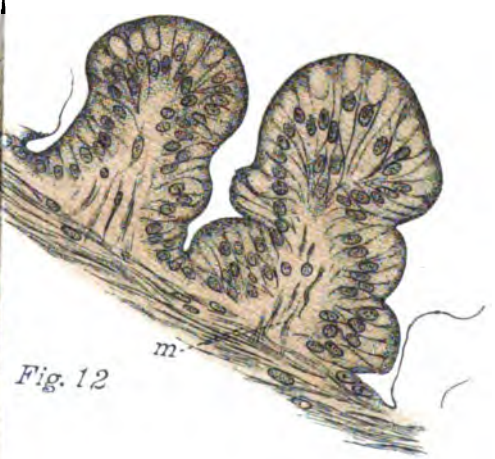


Fig. 12

PECK, del.

Fig. 19 a

Fig. 13

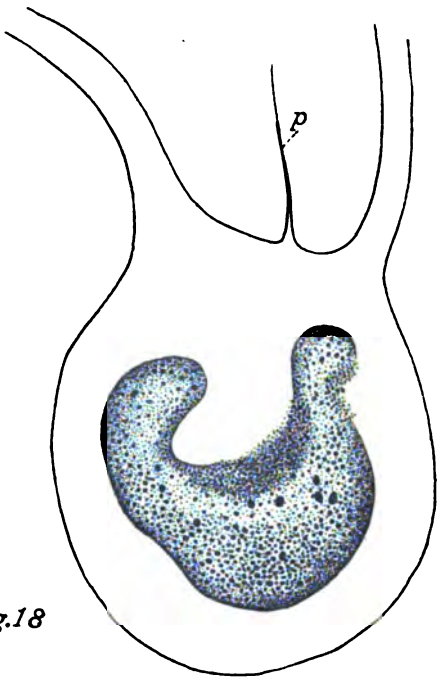
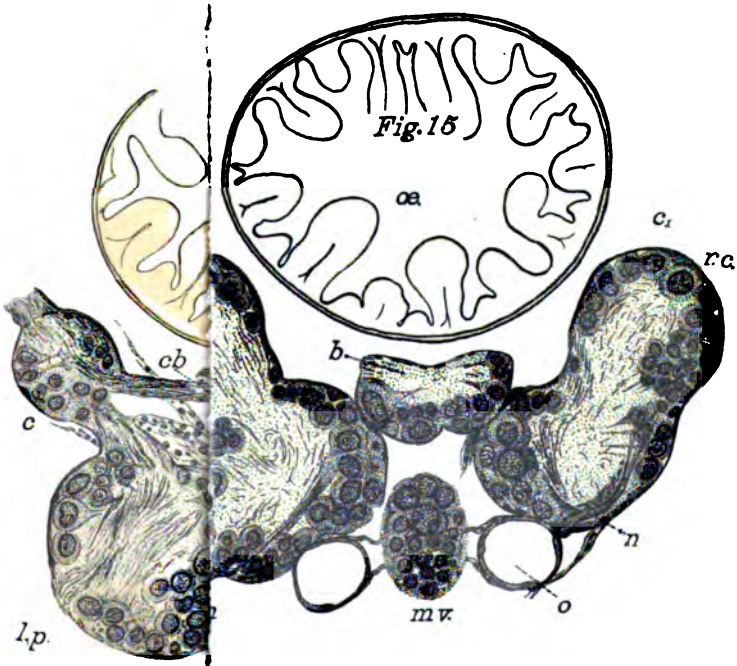


Fig. 18



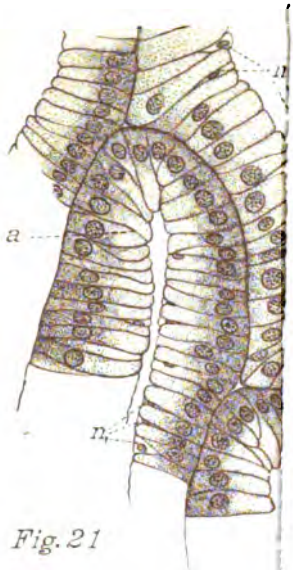


Fig. 21

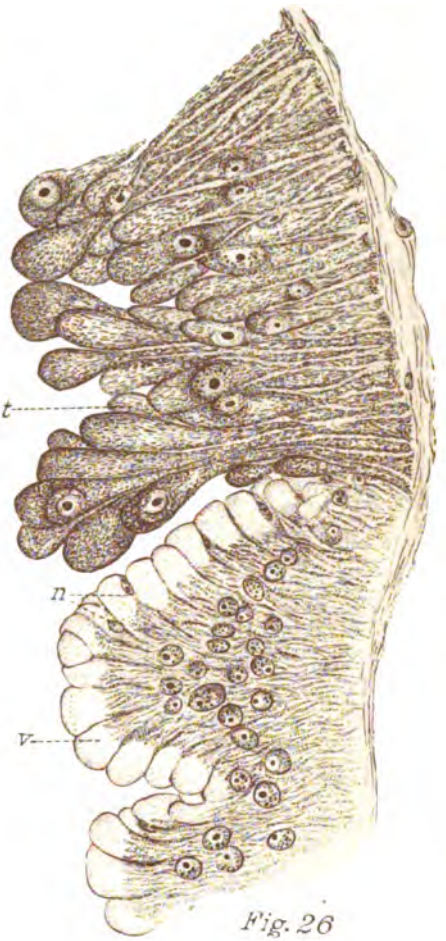
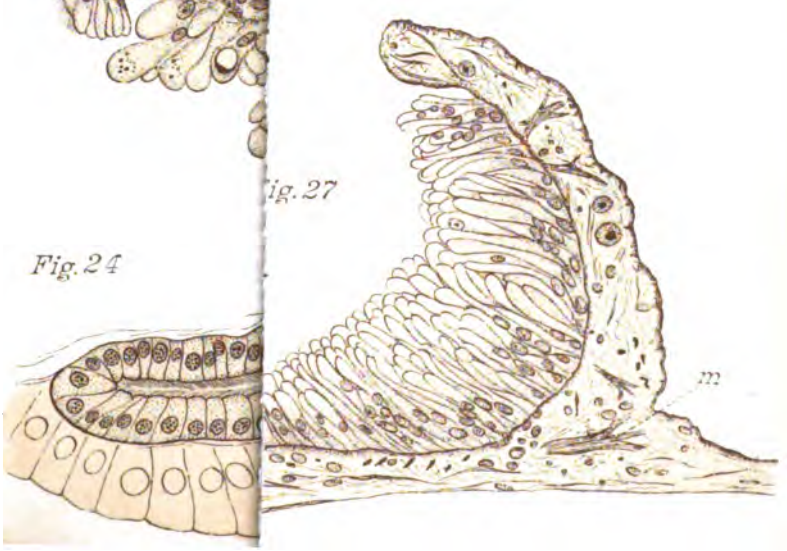


Fig. 26

Fig. 2

ig. 27

Fig. 24



ON THE AMPHIBIAN BLASTOPORE.

By T. H. MORGAN. With Plates XL, XLI, XLII.

An examination of the literature of the embryology of Amphibia will show very many references to the origin and "fate" of the blastopore. Not only are these references due to the importance of the changes which the blastopore undergoes, but are in many cases only passing notices of its condition as seen from surface views or as met with in sections. Out of all this work we ought by this time to have arrived at some definite knowledge relative to the changes which this important structure undergoes, yet such is by no means the case. Some of the most recent papers on the frog's blastopore have only led to confusion and inexplicable positions. The present account applies to three genera of Amphibia, two Anura and one a Urodele, viz. Bufo lentiginosus, Rana halecina and Amblystoma punctatum. The research was done under the direction of Prof. W. K. Brooks, to whom I am greatly indebted. Much of the work was at first carried on with Mr. E. C. Applegarth, for whose assistance I desire to express my obligations.

The embryos were hardened in picro-sulphuric acid and in Perenyi's fluid. In nearly all cases the eggs were cut without staining. The cell-boundaries are thus very distinctly seen, and it appears that this is due to extremely small pigment granules which are found over the periphery of the cells, and form in sections a narrow border to each cell. The stippling in the figures is intended to indicate these pigment granules. Only in exceptional cases can the nuclei be seen without using staining reagents. During the early spring of 1889 I collected for a second time the embryos of Amblystoma, and by means of these I have again gone over that part of the work relating to Amblystoma. The embryos were put into Perenyi's fluid for an hour, removed to 50 per cent alcohol and dissected under a dissecting microscope. In this way I have verified and added to the results previously obtained by surface views and sections, and have interpolated these results amongst those which had been obtained during the winter.

AMBLYSTOMA PUNCTATUM.

The dark cells of the ectoderm gradually grow over and almost enclose the endoderm, which is then seen as a plug circular in outline, surrounded peripherally by the ectoderm. Very soon this circular blastopore begins to elongate (Fig. A, Pl. XLII), and a careful examination of the periphery of the embryo shows at this time a slight indentation, which may be referred to a slight surface depression—the primitive groove. (Fig. A.)

In the next stage (Fig. B) the endodermal plug has disappeared within the blastopore, and the blastopore itself has become even more elongated; the primitive streak is quite conspicuous, and on each side of it a slight elevation of the ectoderm indicates the beginning of the neural folds.

In stage C (Fig. C) the neural folds have become very conspicuous, the primitive streak narrow (not shown in figure). On each side of the blastopore run the neural folds almost to its posterior end.

The next stage is shown in Fig. D. The blastopore is proportionately smaller than in the earlier stages. As seen from without it has assumed in Fig. D a flask shape, with the anterior end corresponding to the base of the flask. From this stage up to and including the next (Fig. E), I have had the best success from dissected and teased specimens.

The posterior end of the embryo, including the blastopore, was cut off, the mass of endodermal cells were removed by means of teasing needles under a dissecting microscope, and the remaining portion examined both from without and from within. In this way my results have been more definite than I could obtain either from external views or serial sections, and all three methods taken together have given very conclusive and satisfactory results.

To return to stage D. Frequently the blastopore is flask-shaped, yet I have found some embryos in which it was simply an elongated slit-like opening. If an embryo at this stage (D) has its upper wall cut away with a sharp razor just above the blastopore, exposing the archenteron, and the lower half be examined with a hand lens, one may easily make out the conditions shown in Fig. D′. In front this archenteron dips down to form a large, deep cavity. Behind it suddenly narrows from

before backwards, but remains wide from side to side, and continues down to the blastopore. This posterior portion of the archenteron is as seen from above semilunar in shape, the result of a great thickening of the wall of the embryo in the middle posterior line around the blastopore.

Passing to stage E we find that the medullary folds have fused almost completely, the only opening to the exterior being at the anterior end of the tube, which is shown by figure E′. This stage is by all odds the most important with respect to the changes of the blastopore. For this reason a side view of an embryo of this age is given in Fig. E‴. Surface views at this time show the posterior end of the blastopore projecting just beyond the enclosed medullary folds as a small opening. Dissections of embryos, aided by teasing, have here given the most satisfactory results. If the posterior end of the embryo be cut off as before, and the mass of endodermal cells, in front of the posterior down-growth of the archenteron, be removed, one sees the inner opening of the blastopore.

Fig. G′ is a view of the inner end of an embryo in the region of the blastopore at the same age as E. The elongated slit-like opening lies surrounded by a mass which projects inward. At one point, however—the posterior—the blastopore projects beyond this thickening, as is shown in the figure. The shape of the blastopore varies in different individuals. That given in the figure is quite common, but the one most frequently met with is a dumb-bell-shaped blastopore, where there are two enlarged openings at the ends connected by a narrower slit-like cavity. Turning the dissected piece over and slightly stretching with teasing needles the walls from side to side, it is easy to throw a ray of light from the mirror of the microscope through the posterior end of the blastopore, which is seen as a small hole just beyond the fused medullary folds. (Fig. E.) With sufficient care one may tease apart the medullary folds at their posterior end, exposing the neural tube and blastopore. This is shown in Fig. E″, in which the medullary folds have been drawn slightly apart and a ray of light thrown up through the blastopore. The blastopore is seen to be an elongated opening which stretches from between the medullary folds, posteriorly just beyond their limit. See Fig. H also.

Fig. G shows this for another individual, which is the same from which G′ was taken. Almost immediately after this stage the whole of the blastopore closes, with the exception of its posterior end, which is seen projecting beyond the medullary folds. At the same time the lumen to the posterior end of the medullary folds becomes obliterated. Just at the stage when the blastopore closed I have dissected off the posterior end of the embryo and cut serial sections. These sections show the method by which the walls of the blastopore come into contact and fuse over the whole of its anterior area, while the posterior part of its opening remains as the anus of the adult.

In stage F the anal end of the blastopore is all that remains. Fig. I is from a dissection of an embryo at a similar stage. The anus next shifts to the under and posterior side of the embryo, as shown in the figures. Serial sections of embryos earlier than stage E add little to what may be gathered from surface views. On each side of the blastopore the walls are greatly thickened, as is shown in Fig. 2, Pl. XL, which is, however, from an embryo at a later stage.

A series of longitudinal-horizontal sections of stage E, which cut the blastopore at right angles, is most instructive. Fig. 1, Pl. XL, is a section just anterior to the blastopore in such a plane. The neural tube is shown by *G*, and the medullary folds have just come in contact above the tube. Under the neural tube is a thick floor, below this a layer of endoderm cells, then the cavity of the archenteron. This cavity is a prolongation of the archenteron, which runs downwards and backwards.

In the next section, going backwards or downwards from Fig. 1, the neural tube is seen to extend inwards towards the digestive tract. This is even more marked in the section following, where only a few cells separate the two cavities.

In the next section, Fig. 2, the two cavities are directly continuous. This condition is found through the next four sections. Then the walls of the neural tube come together, as in Fig. 3, leaving the narrow archenteron beneath.

In Fig. 3 the walls are seen not actually fused but only touching above the archenteron. Such conditions are found through the next fifteen sections (each about 10 μ. thick), when we reach Fig. 4. In this the archenteron has narrowed very

much from side to side, and above it the walls of folds have somewhat separated.

Fig. 5 is the fourth section after Fig. 4 showing the opening of the anus to the exterior. The arrow in Fig. 7 indicates the plane in which these sections were cut.

Figs. 6 and 7 are from two longitudinal-vertical sections of embryos, the first at a stage between D and E. The direction of the anterior end of the embryo is indicated by the arrow (*Ant*). The archenteron is indicated by *D*, and from it a prolongation of its cavity runs downwards and backwards to the blastopore. In Fig. 6 the medullary folds have just met above the blastopore, and the isolated piece *M* is a section through the outer ends of the cells which have touched across or above the cavity of the blastopore. By this arching over of the medullary folds a neurenteric canal is formed which puts the cavities of the medullary folds and the digestive tract into communication, as is shown at *N*. Sections to the right and left (on each side) of this show clearly the arching over medullary folds of the two sides whose point of contact was shown in Fig. 6, and the two sections immediately to the right and left show very distinctly the neurenteric canal. Fig. 7 is a longitudinal-vertical section from an embryo a little older than the last, and corresponds approximately to Fig. E. In it the medullary folds have fused completely over the blastopore. The neurenteric canal is shown by *N*. While this section cuts the blastopore approximately in the middle line it does not cut the medullary folds quite perpendicularly, but the thickness of one section to the right or left. This gives a somewhat exaggerated idea of the thickness of the fusion of the medullary folds, but shows very well the arching over of this continuation of the medullary folds to form through the anterior end of the blastopore a neurenteric canal. The posterior end of the blastopore opens freely to the exterior at *A* as the anus.

The neurenteric canal is very transitory, and consequently easily overlooked. I have cut embryos (in several planes) older than stage E to see if it were possible to find a connection between the neural tube and archenteron, but in no case have I discovered any connection whatever. The cavity of the nerve chord—the neural tube—ends blindly behind in all these later stages, and

although the cells composing the nerve chord are fairly well shown extending far behind the neural tube, I have not been able to make out any lumen to the nerve chord. I do not believe there can be any doubt that the transitory connection between the neural tube and the digestive tract, by way of the anterior end of the blastopore, represents the neurenteric canal. When one sees from surface views (Fig. H) that the medullary folds actually close over the anterior end of the blastopore, and when one sees further that the anterior end is considerably enlarged and rounded, that in most cases the middle part of the blastopore is obliterated by the fusion of its walls, that all these changes seen from the exterior are fully verified by serial sections, I see no ground for any other supposition than that *the anterior portion of the blastopore becomes the neurenteric canal, and the posterior end the permanent anus of the adult.*

Rana halecina.

A series of surface views is most instructive. The medullary folds are from the beginning quite near together as compared with Amblystoma. The groove in between the folds is directly continuous behind into the blastopore. The medullary folds approach and bring about the conditions seen in Figs. J and K, Pl. XLII. In this the groove between the medullary folds is seen to continue even posterior to the blastopore, and at its posterior end there is seen from the surface a dark spot which careful focusing of the microscope will show to be a slight invagination. The blastopore also appears as a black spot just anterior to the last. And, moreover, the posterior dark spot is seen to be a later formation than the blastopore. Connecting these two "spots" there is seen a broad pigmented groove.

A longitudinal-vertical section of an embryo at this stage is shown by Fig. 11, Pl. XLI. The blastopore is seen opening to the exterior at *B*.

Beneath the blastopore there is seen in this vertical section a knob-like prominence projecting upwards. This knob in the sections on each side of the one figured is seen to be continuous with the general walls of the embryo, and is thus not a knob at all but a bridge of cells running beneath the blastopore. Below this there is an invagination from without inwards at *A*, and

there is further a corresponding invagination of the archenteron from within outwards. The two cavities are separated by only a single layer of ectoderm cells. It is also worthy of note that in the region of the plug or band the surface ectoderm is considerably below the general ectodermal surface of the embryo.

Figs. 8, 9, 10, Pl. XL, give horizontal-longitudinal sections through an embryo at the same stage as Fig. 11. The first of these (Fig. 8) is through the open blastopore. Beneath the opening of the blastopore and continuous with it there is seen the posterior continuation of the archenteron, much flattened from behind forwards, but very wide from side to side. Fig. 9 is a section behind or beneath Fig. 8, between *B* and *A* of Fig. 11, and cuts the plug or bridge of cells. A very conspicuous groove is seen on the outside of the section. This groove is continuous with the medullary groove in front and behind into the deep groove shown in the next Fig. 10, which is a horizontal section of the same series through *A* of Fig. 11. In this section (Fig. 10) the external groove is seen to be separated from the archenteron by a single row of ectodermal cells. The archenteron is still quite wide from side to side, and somewhat deeper from before backwards than in the preceding section. Owing to the fact that this anal invagination *A* extends downwards as well as inwards, as may be seen in vertical section (Fig. 11), the sections following Fig. 10 cut the continuation of the groove as a cavity lined by ectoderm and still separated by a single row of cells from the archenteron.

In an embryo somewhat older than the last, horizontal sections show that the medullary folds of Fig. 8 arch over the blastopore and enclose it. See Fig. 13. Thus the neural tube which is also formed by the arching medullary folds comes to open posteriorly by means of this neurenteric canal into the archenteron.

Sections in the same series as Fig. 13 but below it show almost the same condition as we have seen in Figs. 9 and 10, or, in other words, immediately after the closure of the blastopore the archenteron does not communicate for a time with the exterior. Thus without doubt we may interpret the two dark spots seen on the surface at the posterior end of the neural canal as the blastopore anteriorly and the invagination of the anus behind.

In Fig. 12 is a drawing of a longitudinal-vertical section through the posterior end of an embryo which is somewhat older than any of the foregoing stages. In it the blastopore is completely closed over and forms the neurenteric canal *N*, which still communicates with the neural tube above and the archenteron beneath. The connection with the neural tube is only partially shown in this figure, but in the neighboring sections it is clearly seen. Beneath the neurenteric canal the endodermal cells of the posterior wall project somewhat into the archenteron as seen in the figures. This projection plainly corresponds to the plug of previous sections. Beneath this plug (or bridge) the cavity of the archenteron projects somewhat outwards just opposite an ingrowth on the exterior, and the two cavities are separated by a single row of much pigmented ectoderm cells.

The last change which takes place is shown in Fig. 14, in which the final condition has been practically reached. The anus has opened at *An* to the exterior so that the archenteron once more communicates behind freely with the exterior. Above the anus, the part marked *P* in the preceding figures is still to be seen. The neurenteric canal at this stage has completely closed.

Between embryos of the stage of Fig. 12 and Fig. 14 I have several intermediate stages, in some of which the neurenteric canal still communicates above with the neural tube, and in some the anus is seen to communicate with the exterior, so that Fig. 13 does not represent the earliest breaking through of the anus to the exterior, but a little later stage.

I have said that in embryos younger than any here figured the downward extension of the archenteron is separated at its lowest limit from the ectoderm by several rows of cells (see Fig. 15). Horizontal sections show in a fairly conclusive way how, from this condition, the later condition (with a single cell layer separating the archenteron from the exterior) is brought about. It seems that the cells lying between the ectoderm without and the endoderm within gradually draw away or migrate to the sides, and at this same time the outside groove deepens and the archenteron extends outward, so that ectoderm and endoderm come nearer together. Next the lower layer cells of the ectoderm begin to pull away from the same point, and then the endoderm cells

show a similar migration. This is shown in Fig. 21 for the toad. I have no direct evidence that the remaining one cell layer of epiblast separates in a similar manner; yet from the fact that the first communication of the archenteron to the exterior is a very narrow opening and gradually widens, and from the migration of the cells which were just beneath the ectoderm, mesoderm, endoderm, I am inclined to believe that the archenteron is put into communication with the exterior by the migration of the remaining epiblast cells away from a central point.

Bufo lentiginosus.

In my study of the toad-embryos I have had recourse largely to horizontal and longitudinal sections, as these best show the relationship of the blastopore to anus and neurenteric canal. By the arching over of the medullary folds there is formed a large neural tube.

A horizontal section through the posterior part of an embryo is shown in Fig. 16. This is taken at a time when the medullary folds are just meeting above. The large neural tube is seen above this; the folds are on the point of fusing together, and immediately above the tube the process of fusion is carried so far that a few cells are in actual contact.

Fig. 17 represents the section following Fig. 16. The floor or base of the neural tube is entirely absent, or, in other words, at this point the medullary folds have arched over and met above the blastopore. In this section also a few cells above the neural tube are in actual contact. Fig. 18 is the section following Fig. 17, where the conditions are very similar to the last, except that the medullary folds are not in actual contact above this part of the blastopore, which would still communicate with the exterior. The communication is even more marked in the next four sections. After this the neural tube and neurenteric canal are entirely absent, and the cavity of the archenteron is separated from the exterior by the thick wall composed of the three embryonic layers. (See Fig. 15 for the frog.) In these sections (eight in number) there is seen in the posterior middle line a distinct groove, and on each side of this furrow the body walls of the embryo are somewhat thicker than elsewhere, and at the base of this groove the cells are much more richly pigmented than over

the general surface. Horizontal sections in stages younger than the embryo just figured show the blastopore opening freely at the surface, with a thickened combination of the medullary folds on each side. In Fig. 19 I have figured a horizontal section of an embryo a little older than that in Figs. 16, 17, 18.

In it the medullary folds are seen completely fused above the blastopore, and the neural tube thus comes to communicate freely with the archenteron through the neurenteric canal *N*. Sections above and anterior to this show simply the fused medullary folds above the large neural tube. Sections behind the one figured show an outer pigmented groove running through twelve sections. Beneath this groove the body walls of the embryo are quite thick, but in the eleventh and twelfth this thick wall grows thin, composed of a single layer of cells, and these cells are even more strongly pigmented than those in the groove with which it is continuous. A vertical longitudinal section through an embryo at this stage is shown in Fig. 20, and exhibits clearly these relationships. In this vertical section there is seen to be no communication of the archenteron with the exterior. At *N* the blastopore has been closed over by the medullary folds *M*, which in this embryo have perfectly fused. At *N* the neurenteric canal is indicated opening above into the neural tube and below into the archenteron. Between *M* and *A* in the figure is seen the thick portion of the wall of the mesenteron which was spoken of in the horizontal sections. At *A* the posterior wall of the archenteron is extremely thin—about one cell in depth—and later stages show this to be the place at which the anus opens to the exterior. If we examine sections of embryos younger than this last in the region of *A* we find that both longitudinal and vertical sections show the wall in this region several layers of cells in thickness. Under the ectoderm come two or more rows of mesoderm cells, and then a single row of endodermal cells. Embryos between this condition and that indicated by Fig. 20 show intermediate stages of development. (See Fig. 21.) Here there is seen to be a general migration of the mesoderm and endoderm from the middle line over a limited area.

Stages later than Fig. 20 show that the anus has opened at the point *A* just beneath the thickened mass situated above *A* in

Fig. 20. Even in the toad, in which from the first the neural tube and neurenteric canal are very large, I cannot trace the persistence of the neurenteric canal to as late stages in development as Goette's figures show it to remain in Bombinator.

Review of Literature.

I cannot hope to give here a complete review of the literature of the blastopore of Amphibia. All that is attempted is a brief abstract of the principal opinions which have been held especially within the last ten years, and a few notices of papers containing accounts of the blastopore in other vertebrate groups. My object is not to give a complete bibliography, but to bring together those facts, a discussion of which will follow in the next section in connection with the forms just studied.

1. Goette (1875) found in the development of Bombinator that the blastopore, or anus of Rusconi, becomes narrowed from side to side as the animal develops and is reduced to a furrow. Then the walls of the blastopore approach first from without; that is, arch over the blastopore. Later their walls come into contact. The neural tube narrows towards the tail and opens into the arched-over blastopore. The proctodaeum is formed later by an invagination of ectoderm.

2. Balfour writes that in the Elasmobranch fishes the medullary folds unite to form a canal, and "this canal becomes continuous with the alimentary tract," or, in other words, the blastopore (at its anterior end) forms a neurenteric canal. "This communication between the neural and alimentary canals .... is a feature of great interest, which ought to throw considerable light upon the meaning of the neurenteric canal."

3. Clarke ('78–'79) has described the external changes in the development of Amblystoma punctatum; there is little said, however, as to the relation of the blastopore to medullary folds or anus. He speaks of the anal end of the medullary fold, and one would infer from his account and from his figures that the blastopore probably formed the anus. No mention is made of a neurenteric canal.

4. Scott and Osborn ('79), on the development of the newt (Triton taeniatus), say, "As the [medullary] folds enclose the blastopore, which remains open till a much later period, the

sinus [rhomboidalis] gives a communication from the exterior to the alimentary canal; when the sinus closes there is still a communication between the neural and alimentary canal." I do not find any account of the formation of the permanent anus.

5. Balfour ('79), in his Embryology, Vol. II, makes the following statements, which are relevant to the present subject: "It is hardly credible that the existing anus [in Chordata] can have been the original one. Although, therefore, it is far from easy on *philosophical principles involved in the Darwinian theory to understand the formation of a new anus*, it is nevertheless necessary to believe that the present vertebrate anus is a formation acquired within the group of the Chordata and not inherited from some older group .... The neurenteric canal is, however, the remnant of the blastopore. It follows therefore that the vertebrate blastopore is probably almost if not identical in position with the primitive anus."

6. Scott ('80), in a short note in the Zoologische Anzeiger, says that in the lamprey the blastopore is arched over by the medullary folds and forms the canalis neurentericus, and that the anus is a new formation.

7. Bambecke ('80) believes the blastopore in Triton disappears before the formation of the lyrmudeal folds.

8. Hatschek ('81) shows that in Amphioxus the blastopore becomes the neurenteric canal and the anus is a new formation.

9. Strahl ('82) says that the neurenteric canal is formed in the middle, not at one end, of the primitive streak in Lacertilia.

10. Gasser ('82). I have not had access to this paper, but from reviews I gather that he derives the anus from the blastopore, and that there is in the newt a partially solid neurenteric canal.

11. Weldon ('83), in a note on the development of the lizard, points out that the anus is formed at some point along the primitive streak, or that the "anus is in the position of a part of the blastopore."

12. Whitman found in "a rare form of the blastoderm of the chick" that the primitive streak was continued out beyond its usual length, and runs through the area opaca to terminate in the marginal notch, showing conclusively that the primitive streak in birds to be a remnant of the line along which the blastopore closes.

13. Schultze ('84) believes that in Rana fusca the blastopore, by the closing over of the neural folds, becomes the canalis neurentericus, and that the anal invagination is formed behind the thick posterior end of the medullary thickening.

14. Sedgwick ('84) advanced an hypothesis "on the original function of the canal of the central nervous system of vertebrata." He suggests that the ancestral vertebrate had a groove extending "along the whole length of the middle dorsal (or ventral?) line of the body, the nervous system being placed in the deeper layers of the epidermis of this groove." This groove became converted into a canal open before and behind.

"The function of the groove was, in my opinion, partly respiratory and partly protective."

"How it [the tube] *acquired its opening into the alimentary tract is perhaps hard to understand.* The discussion of this question involves the discussion of a still more difficult question, viz. the relation of the permanent anus to the blastopore. I may, however, point out here that development points to the fact that the blastopore was placed *within the medullary plate, and that therefore on the conversion of the medullary groove into a canal*, the alimentary canal would open into the hinder end of that canal *and the two tubes would open to the exterior together posteriorly.*" He thinks that water entered the neural canal "by the anterior pore, was driven through it by cilia, and at the hind end passed through the neurenteric canal into the alimentary canal and so out by the anus."

15. Sedgwick ('84), shortly after the above hypothesis was advanced, instituted in a brilliant paper a comparison between the primitive gastrula mouth and anus. This hypothesis is so well known that a mere reference will suffice. In that part of his paper devoted to the vertebrate blastopore he says for the newt (T. cristatus) that "in this animal the blastopore appears not to close, but to persist as the anus."

16. Hatschek ('84) says that in the development of Amphioxus the anterior end of the neural tube—the neuropore—is pushed during development to one side, the left, and remains open so that the neural tube communicates freely with the surrounding water. This neuropore persists in the full-grown animal and serves as an entrance for water into the medullary tube.

17. Johnson, A. ('84), verified fully Prof. Sedgwick's observations as to the connection of blastopore and anus of the newt. She gives a review of some of the previous papers, and calls attention to the fate of the blastopore in the different groups of vertebrates.

18. Van Wijhe announced later than, but independently of, Prof. Sedgwick, the hypothesis that between mouth and primitive anus of the ancestral vertebrate the neural tube formed a water passage, whose posterior end fused with the blastopore to form a blastoneuropore through which the water-stream and faecal matter were discharged. Later the blastoneuropore closes in part, and the water passes down the neurenteric canal, and together with the faeces, out of the permanent anus, which has been formed at about this time. Later, both neuropore (anteriorly) and blastoneuropore (behind) became obliterated.

19. Caldwell ('84) has a short note in *Nature* on Ceratodus. He found segmentation holoblastic. "*Part* of the blastopore remains open and persists as the anus." "The stages up to hatching closely resemble those of the newt Amblystoma." The two statements taken together and in the light of what does take place in Amblystoma are very interesting.

20. Spencer ('85). From his article on the Early Development of Rana temporaria I quote: "Thus both sections and external views lead us to the same conclusion that in Rana as in Triton the blastopore is transformed into the permanent anus .... that in Triton no connection exists between the neural and alimentary canals, whilst such a structure is well marked in Rana."

21. Cunningham ('85) says: "With regard to the primitive anus I cannot accept Sedgwick's idea that the present anus is the same structure, nor do I accept the proof brought forward by Miss Johnson on the point as far as regards the newt." "The blastopore is the primitive anus, but does not coincide with the actual anus." The primitive anus he believes was closed over by the medullary folds and became the neurenteric canal, which he believes is "never altogether unrepresented in vertebrate embryos."

21½. In a paper published ten months later, "on the relations of the yolk to the gastrula in Teleosts and other vertebrate types," he seems to some extent to have changed his scepticism in regard to the permanent anus and makes the following statement:

"But Sedgwick and Miss Johnson have lately attempted to show that in Triton part of the blastopore extends round to the ventral surface . . . . If the proposition is correct that the posterior end of the blastodermic aperture in the newt remains open, the question immediately arises: Is the whole edge of the blastoderm in the newt invaginated? The answer to which question cannot be found in Miss Johnson's figures. If it is not, then the blastopore (primitive) and the edge of the blastoderm in the newt are not the same thing. If it is, *then it may prove true that the anus is derived from the posterior end of the blastopore*, and has passed from an original neural position forwards on the ventral surface by a continuous process during ancestral history, without ever closing up . . . . This would mean that the closure of the medullary canal posteriorly divides *the open part of the blastopore into two parts, one forming the neurenteric canal, the other remaining open to the exterior and persisting as the anus.*" This supposition states exactly what takes place in Amblystoma. I did not meet this statement until all the previous sections of this paper had been written, and so found out the facts in Amblystoma without any knowledge of this possible prophecy.

22. Shipley ('85) says in Petromyzon the nerve chord is formed by delamination; later a cavity appears in it. The neural folds do not grow over and enclose the blastopore, as Scott affirmed, but the blastopore becomes the anus. There is a solid post-anal gut, and "it is perhaps worth while to point out that it lies at what was originally the anterior lip of the blastopore."

23. Johnson and Sheldon ('86) have some further "notes on the development of the newt." In addition to the fact that the blastopore becomes the anus, they find a solid diverticulum of the alimentary canal, which no doubt represents the neurenteric canal, and this canal they believe to be always solid in the newt.

24. Durham ('86) believes that the blastopore persists as anus in Rana, but found one series in which there is no blastopore opening, and this he regards as abnormal. He finds there was a well marked communication "between the alimentary tract and the neural canal without the intervention of the blastopore," but does not suggest how such a communication has been brought about. It seems to me that his abnormal embryo is a most normal form and to show the stage when the blastopore has closed and a new anus not yet formed.

25. Haddon ('87) in his Embryology says, "Most embryologists consider the anus of vertebrates to be a new structure, but Sedgwick regards it as the posterior extremity of the primitive blastopore"; and in another place, when speaking of Cyclostomes and Amphibia, "in these groups it appears that the blastopore persists as the anus, consequently it was behind the post-anal gut (solid in the newt), which was imagined to extend between the closed-over blastopore; and the new anus is merely a ventral extension of the neural canal owing to the growth of the trail taking place above the blastopore."

26. Sidebotham ('88) gives in the *Quarterly Journal* for July, 1888, one of the latest accounts. He says, in the frog, as the neural folds grow up to meet each other they do not enclose the blastopore, but reach only as far as its dorsal anterior rim. The blastopore closes and a new anus is formed by a proctodaeal invagination.

I do not get from his account a clear idea as in what way the blastopore completely disappears, but he seems to believe that it does not at any rate form the neurenteric canal.

27. Before completing my work on the toad and frog I published ('89) a preliminary note on the fate of the blastopore—chiefly in Amblystoma. From my study of the embryos of Amblystoma I concluded that there was a possibility of the blastopore in other forms becoming the neurenteric canal. Further work on the toad and frog has, I believe, found this supposition true.

28. Giacomini, "Neurenteric Canal in the Rabbit." I have only seen the abstract in the Journal of Royal Micro. Soc. ('89). Here it is stated that he is inclined to believe the hypothesis that the ideal, ancestral medullary-canal and gut had a common opening, "the blastoneuropore."

Comparisons and Conclusions.

Comparisons.

From the brief review just given I think we may in general draw the following conclusions as to the fate of the blastopore:

1. In some forms it becomes altogether or in part the neurenteric canal. Newt (Scott), Frog (Goette and others), Elasmobranch, Bird, etc.

2. In some forms it becomes the anus. Newt (Sedgwick), Frog, Petromyzon (Shipley), Ceratodus (Caldwell).

3. In some forms it entirely closes and a new anus is formed. Frog.

4. The possibility of its becoming both neurenteric canal and anus has been suggested. This last (4) I believe to be the condition at the present time in Amblystoma. If this is accepted it seems to me to give us a definite point from which to start towards an explanation of what has been discovered in other forms. If we accept the fact that in at least one living animal the blastopore gives rise to both neurenteric canal and anus, I think most of the previous observations are fairly well explained.

It may be asked, what evidence is there that this is an ancestral condition? Why do we not find similar phenomena in forms lower than the Urodele Amphibia? I have no direct evidence to offer against these objections, and can only meet them by the following propositions.

In some of these lower groups, as the Cyclostomes and Teleosts, in which a solid neural chord is formed, we may regard this (and I believe it is generally so regarded) as a secondary phenomenon, and to be referred to ancestral forms whose chord was made by a rolling in of ectoderm. And again, on the other hand, in such ancestral forms as the Salachians and Dipnoi we have changes in their development which will support a presumed ancestral condition for the blastopore of Amblystoma. In the Salachians we know the blastopore in part to become the neurenteric canal, while in the Dipnoi—and this is most important—we have a single observation which shows that in Ceratodus the posterior part of the blastopore becomes the permanent anus, and further, that the general development resembles that of Amblystoma. So far as I know no observations have yet been made as to the neurenteric canal in Ceratodus, yet it seems to me there is a good deal of possibility that the anterior end of the blastopore may be here found to form a neurenteric canal. Even further we see that in the small living group, Amphibia, we have reliable evidence that in some forms the blastopore functions as neurenteric canal, and in other closely related animals it forms the anus.

I believe these propositions give good evidence to support the

view that in Amblystoma we have found a form which retains during its larval development for a short period the ancestral conditions of the blastopore. Starting with it as the ancestral form and working backwards, we may see if it will explain the facts. We have difficulty, however, in getting at these known facts; still it seems to me the first three propositions given above must be admitted, although for some particular form this may not be true.

1. In those animals in which the blastopore becomes the neurenteric canal there is no difficulty, especially as it has been shown that in some of these same forms the anus opens later in the primitive streak. The lizard is an example.

2. Nor does the second case present any difficulty, and in this connection such a statement as "it is perhaps worth while to point out that a solid post-anal gut lies at what was originally the anterior lip of the blastopore," explains itself on our hypothesis.

That the neurenteric canal is never open in some forms, but remains a solid chord of cells, we must admit as a probability, but in some cases in which this has been affirmed I think that future work may show that for a very short period the conditions seen in Amblystoma may present themselves. Still Amblystoma does show an intermediate step between an open neurenteric canal and a solid mass of cells at the anterior end of the blastopore.

3. This brings us to the third case, in which the blastopore closes and a "new" anus is formed. The closure of the blastopore has just been sufficiently discussed, and with the second part it seems to me, from a study of the frog and toad, we have no difficulty.

The new anus is merely a backward extension of the original blastopore, and in this connection the deeply pigmented furrow running from the old blastopore to the "new" anus is worthy of notice. Further, the method in which the new anus breaks through to the exterior lends support to the case. I see no other interpretation than that this migration of cells away from a central point is merely the opening of the posterior part of an elongated blastopore. Thus all the evidence points to the fact that the original blastopore gave rise to both the neurenteric canal at its anterior end, and at its posterior to the permanent anus.

Conclusions.

From the facts stated in the first part of the paper it seems that the blastopore in Amblystoma was situated in the posterior part of the medullary groove and partially surrounded by a continuation of its walls on each side; that it was then overarched in its anterior part by a continuation of the medullary folds; that on account of its elongation its posterior end escaped this closing over; and that by the shutting in of the medullary folds the digestive tract came to communicate with the neural tube by the anterior end of the blastopore, and with the exterior by the posterior end of the blastopore.

Consider these facts in connection with the well known phenomenon of the persistence of the neurenteric canal in animals in which at present we can conceive it to have no function, also that in one ancestral animal—Amphioxus—the ciliated neural tube communicates directly by the neuropore with the surrounding water, and I can see no escape in the present state of our knowledge from part of the hypothesis which Sedgwick has announced.

It seems to me that the following hypotheses or suggestions may at least tentatively be proposed (with one exception—of the exact relationship of the neural tube to the blastopore—it differs little from Sedgwick's hypothesis concerning the origin of the neural tube), that in Amblystoma we have in the behavior of the blastopore the changes which have in general taken place in the phylogeny of that organ.

We have seen that the anterior end of the blastopore has been caught in by the general closing over of the medullary groove. At first I was inclined to believe this purely mechanical, but from the very *great persistence* of this anterior end in many animals as the neurenteric canal, and from the fact that the blastopore walls actually *fuse behind this* neurenteric canal, I must believe the neurenteric canal to be a rudimentary organ which has had at some time an important function. The neurenteric canal has remained pretty stable in position; the anus has wandered far from its original station.

It seems, then, probable that at the same time that the medullary groove was more and more shut in to form the neural tube, the blastopore became also partially arched over by a continuation of the medullary folds.

This might interfere with the free passage of excreta from the digestive tract to the exterior. To avoid this difficulty we have the survival of an elongated blastopore. And now by the growth of the tail the anterior and posterior end of the blastopore separated farther and farther from each other, as indicated in the ontogeny.

I have purposely avoided any mention as to the neural tube forming a water passage. That this came to be its function seems possible from the facts above given. If a nervous ciliated tract came gradually to sink beneath the general surface—let us say for protection—we have the beginning of the neural groove. Such a migration of a sensitive area we see in the nose. That water should still enter this medullary groove is self-evident, and that when even later the groove became entirely closed over to form a tube, that it still may have communicated with the surrounding water is possible from the living example of Amphioxus. Neither does it seem to me improbable that by means of the cilia the water should be driven along this tube, by means of which the nerve cells may either have received stimuli from the surrounding water, or served to aerate the nervous system, or both.

On account of the arching over of the blastopore this stream of water must have entered the digestive tract by means of the neurenteric canal, and thence passed out to the exterior through the posterior end of the original blastopore; and as this posterior part or anus moved further and further away from the neurenteric canal and the intermediate part became closed, we can see how the neurenteric canal came to have an important function and hence its remarkable persistence. That the anus is, in animals higher than the Amphibia, a much later formation than the neurenteric canal is easily explained by retardation in development in animals in which during embryonic life it had no function.

Mr. Wightman has demonstrated to me in the neural tube of adult frogs the ciliated epithelium in the living condition, and further by the addition of suspended carmine granules these cilia are seen to drive the particles *towards the tail*. That a rudimentary organ should remain through thousands of generations in an active moving condition seems most improbable, yet no

secondary function has so far as I know been suggested for these cilia. At any rate the fact that these cilia still drive currents backwards, from brain to tail, is most interesting in this connection.

BALTIMORE, *May* 18, 1889.

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REFERENCE LETTERS.

A, anus.
B, blastopore.
B', groove behind blastopore in Anura.

D, archenteron.
G, neural groove or tube.
M, medullary folds.
N, neurenteric canal.
P, plug or bridge of cells.

EXPLANATION OF PLATES.

PLATE XL.

FIG. 1.—Longitudinal-horizontal section through the posterior end of Amblystoma at stage E. Camera drawing.

FIG. 2.—Second section in series of the longitudinal-horizontal, following Fig. 1.

FIG. 3.—Fifth section, following Fig. 2.

FIG. 4.—Sixteenth section, following Fig. 3.

FIG. 5.—Fourth section, following Fig. 4.

FIG. 6.—Longitudinal-vertical section of Amblystoma at stage between D and E.

FIG. 7.—Longitudinal-vertical section of Amblystoma at stage E.

FIG. 8.—Longitudinal-horizontal section through the blastopore of Rana halecina.

FIG. 9.—One of this same series immediately following Fig. 8.

FIG. 10.—One of this same series through the anal invagination.

PLATE XLI.

FIG. 11.—Longitudinal-vertical section of Rana halecina, showing blastopore and anal invagination.

FIG. 12.—Longitudinal-horizontal section of Rana halecina, showing closing over of blastopore to form neurenteric canal.

FIG. 13.—Longitudinal-horizontal section of Rana halecina after the fusion of the medullary folds.

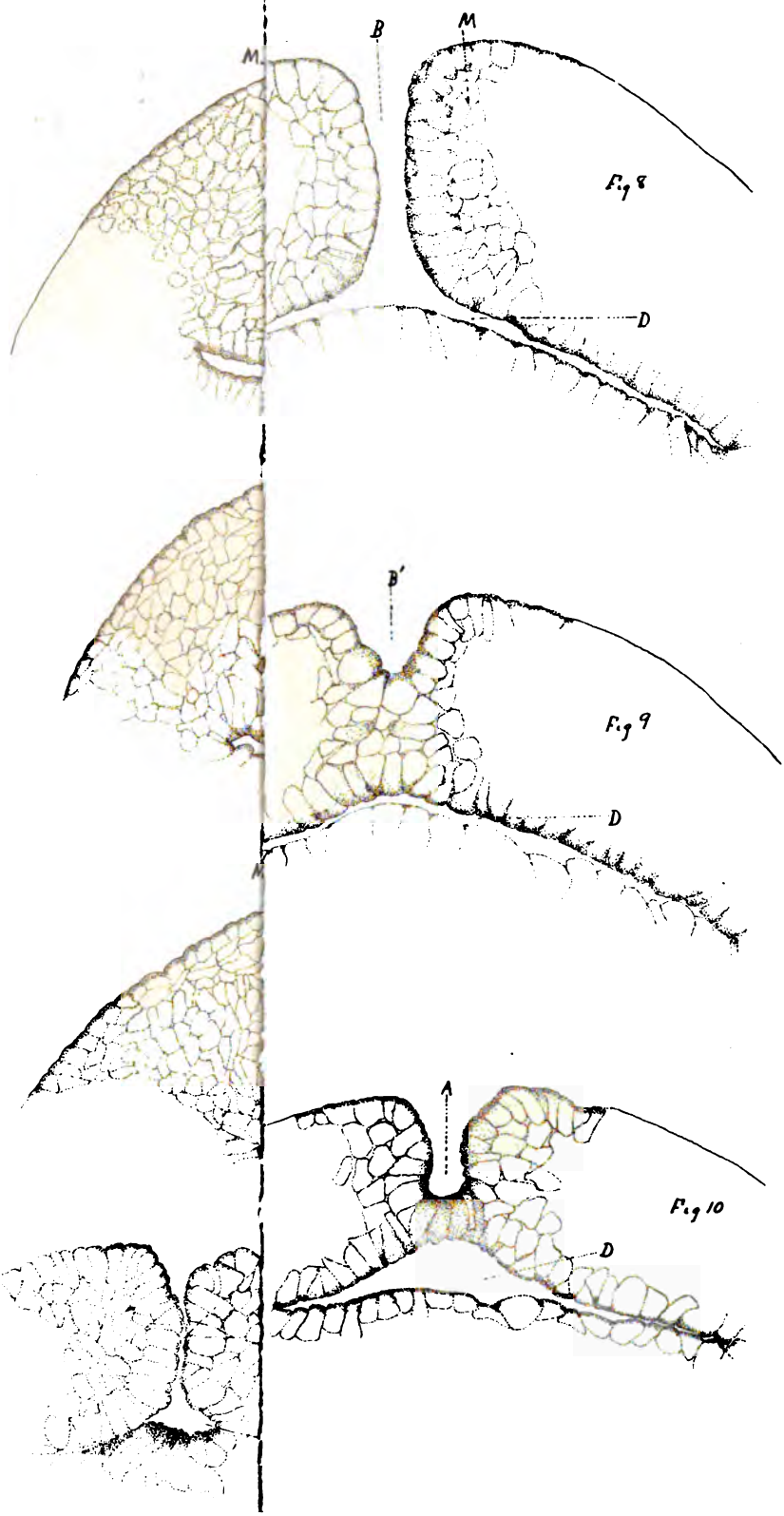
FIG. 14.—Longitudinal-vertical section through posterior end of Rana halecina, showing permanent anus. The neurenteric canal has disappeared.

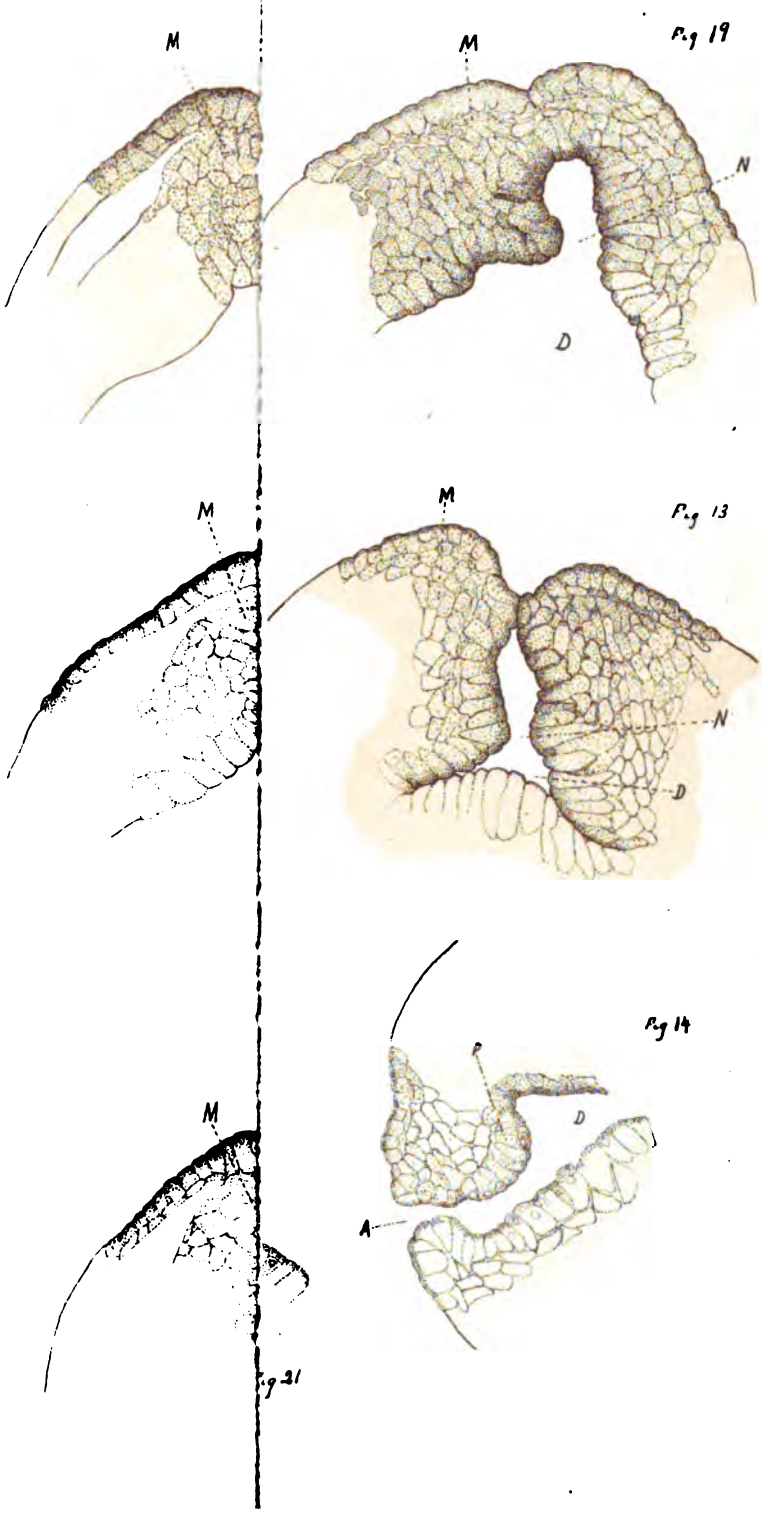
FIG. 15.—Longitudinal-horizontal section through anal region of Rana halecina at a stage before the medullary folds have closed over the blastopore.

FIG. 16.—Longitudinal-horizontal section through the posterior end of Bufo lentiginosus.

FIG. 17.—Section in same series, following Fig. 16.

FIG. 18.—Section in same series, following Fig. 17.


M
B
M
Fig 8
D
B'
Fig 9
D
M
Fig 10
D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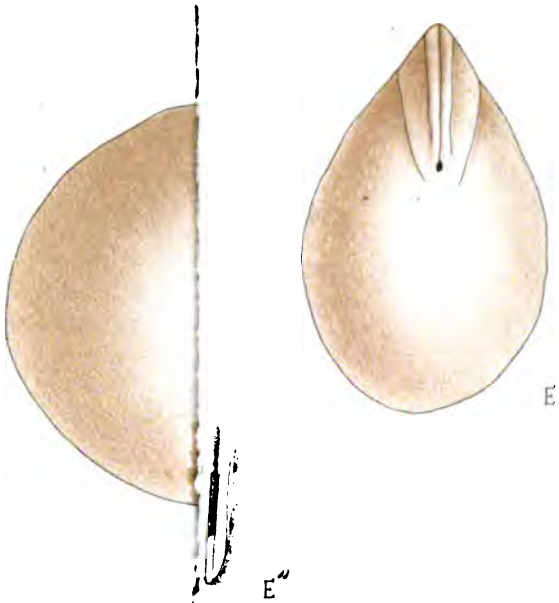
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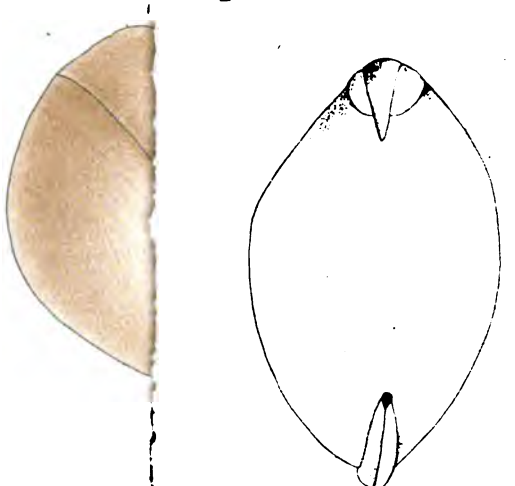
F

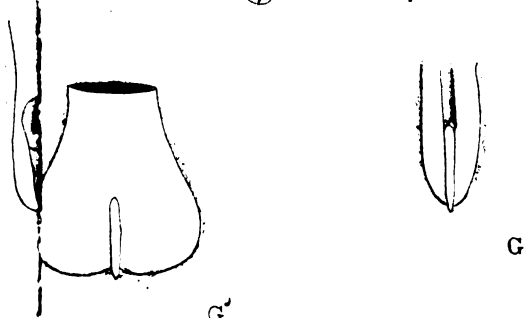
G
G'

FIG. 19.—Longitudinal-horizontal section through posterior end of Bufo lentiginosus, showing medullary folds to form neurenteric canal.

FIG. 20.—Longitudinal-vertical section through Bufo lentiginosus at same stage as Fig. 19.

FIG. 21.—Longitudinal-horizontal section through anal region of Bufo, to show migration of cells from middle line to form the anus.

PLATE XLII.

FIG. A.—Surface view of Amblystoma, endodermal plug still appearing at surface.

FIG. B.—Surface view of Amblystoma.

FIG. C.—Surface view of Amblystoma.

FIG. D.—Surface view of Amblystoma.

FIG. D′.—Dissection of embryo at stage D, the upper wall being removed to show digestive tract.

FIG. E.—Surface view of Amblystoma when medullary folds have just fused.

FIG. E′.—Surface view at anterior end of medullary folds, to show opening to exterior.

FIG. E″.—Blastopore seen from without; the medullary folds have been dissected apart.

FIG. E‴.—Side view of same individual.

FIG. F.—Surface view of ventral side of Amblystoma.

FIG. G.—Dissection of embryo at stage E, to show blastopore as seen from without.

FIG. G′.—Dissection of embryo at stage E, to show blastopore from within.

FIG. H.—Dissection of embryo at stage E, to show dumb-bell shaped blastopore.

FIG. I.—Surface view of anal end of embryo at a stage later than F.

FIG. J.—Surface view of Rana halecina just before medullary folds close.

FIG. K.—Surface view of anal region of Fig. J, to show the blastopore, the anal invagination, and the groove connecting the two.

ON A NEW ACTINIA, HOPLOPHORIA CORALLIGENS.

By HENRY V. WILSON, Ph. D., Bruce Fellow in the John Hopkins University. With Plate XLIII.

The actinia described in this paper was found by me, July 29, 1888, on the small reef which fringes the shore of No Name Key, Abaco, Bahamas. It was discovered in a hole on the under surface of a piece of a green, porites-like asteroid coral which is very abundant on Bahama reefs. The hole extended about half an inch into the corallum, and was freely open to the sea. Though I remained six months in that part of the Bahamas and examined many pieces of coral with the hope of finding more specimens, I never got another. The name I have given the animal signifies the coral-inhabiting weapon-bearer.

1. *Superficial Description.*

Figs. 1 and 2 give two views of the living animal (magnified twenty times). In the lateral view the column is shown extended, but sometimes the animal flattens to a cake-like shape. The twelve long tentacles are of nearly the same size, though six of them belong to one cycle and six to another. The remaining tentacles are much smaller. The tentacles, which are very soft and flexible, are not capable of retraction, though they can shorten their length very much. Just beneath the circle of tentacles are four large and conspicuous organs, which give the animal a most bizarre appearance. Sections show that these organs are diverticula of the gastro-vascular cavity, and are stinging weapons. In the figures (1 and 2) they are drawn at their full size, but often they will contract to one-third of this size.

Color.—The base is white. The column, which is smooth, is a pale brown. The tentacles likewise are pale brown, tipped with white, and the twelve large ones are furnished with an incomplete spiral stripe of white, which sections show to be a spiral band of nettle cells. The peristome is blue with white spots. The œsophagus is a yellowish white. The upper part of the stinging organs, marked *n. b* in the figures, is a bright white.

The rest of the organ when expanded is of a brown color tinged with blue; when contracted it turns decidedly blue. The variety of color and the delicacy of tint make this anemone a very beautiful little animal.

The hole in the corallum which Hoplophoria had chosen for its home was somewhat tubular, with a rather small mouth. The little actinia attached by its base to the far end or to one side of the cavity, would protrude its tentacles and stinging organs out into the surrounding water, in which position the stinging organs were very noticeable. After I had freed its foot from the corallum the animal still endeavored to stick to the wall of the hole by means of its tentacles, which are very adhesive.

2. *Anatomy.*

The general arrangement of parts, illustrated by the diagram, Fig. 3, is on the common Hexactinian type, and offers nothing unusual. There are only six pairs of complete mesenteries, answering to six of the twelve larger tentacles. The remaining six large tentacles correspond to incomplete mesenteries of the second order (2, Fig. 3). Between each two large tentacles are three small ones. The middle of the three (3) corresponds to a pair of mesenteries of the third order. The first and second pairs of mesenteries have the customary muscle plates, but the twelve pairs of the third order, which are very small, are without them. The first, second, and third cycles of tentacles are ento-coelic: each is derived from the space between the two mesenteries of a pair. The twenty-four tentacles of the fourth order (4, Fig. 3) on the other hand are exocoelic: each is derived from the space between two adjacent pairs of mesenteries.

The mesenteries of the first and second orders all have filaments; those of the third are too small to have any. The filaments are typical Hertwig filaments, their upper part being trifid, their lower simple, in cross section. Fig. 4 is through three pairs of mesenteries of the several orders. Those belonging to the second order are cut in the upper part of their course, and their filaments consequently show a middle glandular stripe together with lateral ciliated stripes. Those of the first order are cut lower down, and the filaments here have only the middle stripe. There are no acontia.

In passing I would like to mention that the filaments of two other Bahamian forms, Cereaetis and an unknown genus, are also constructed on the Hertwig type. Indeed it seems that actinias generally have such filaments. The corals, on the other hand, it would appear, have simple instead of trifid filaments. At least I have found this to be true in Manicina, Madrepora, and Orbicella, and from the figures of filaments given in recent coral papers I infer that it is widespread. This difference in the structure of such important organs ought to be taken account of in any speculations regarding the relationship between corals and actinias. The great similarity between Madreporarian corals and Hexactiniae (common actiniae as distinct from Ceriantheae, Zoantheae, Edwardsiae, etc.) leaves no doubt of their near relationship. But Hertwig (Challenger Report of the Actiniaria, p. 21) is even inclined to believe that the separation of forms with a skeleton from those without any may be unnatural —that the various families of corals may be more closely related to special actinian families than to each other. In other words, he advances the suggestion that members of several Hexactinian families have independently acquired a skeleton, and that the group of corals is therefore a heterogeneous assemblage of forms belonging really to various Hexactinian families, though all provided with a skeleton. A strong argument against this view of the Madreporaria is, I think, afforded by a study of the mesenterial filaments. Porous and aporous corals alike have simple filaments, but actiniae have trifid filaments.

The single specimen of Hoplophoria that I found was a female. The ovaries are developed on but four mesenteries, which belong to the primary cycle. Moreover, if one pair of the directive mesenteries be assumed to be ventral and the other to be dorsal, each of the four fertile mesenteries will be the dorsal member of its own pair (Fig. 3). The size of the ovary, as is seen in the true section, Fig. 4, is small.

In Fig. 3, two of the four stinging organs are shown in their proper position. They are diverticula of the spaces included between mesenteries of the second order. The probable position as judged by surface views, of the other two organs, is indicated by the question marks (?) on the opposite side of the diagram. The reason for my doubt on this point is that, having but a single

specimen, I was forced to cut off two of the stinging organs in order to get transverse and longitudinal sections of them, and at the same time have the column of the animal for a series of transverse sections. The diverticulum which constitutes a stinging organ resembles in general that which forms a tentacle (Fig. 4). In one important respect, however, there is an obvious difference. Two tentacles (marginals) are never derived from the same intermesenterial chamber. But the chamber to which each stinging organ belongs gives rise immediately above it to a tentacle (one of the six secondary tentacles). See Fig. 6, a vertical-longitudinal section.

In the living animal (Figs. 1 and 2) the back of the stinging organ has a brilliant white area, which is very well defined. Transverse (Fig. 5) and longitudinal (Fig. 6) sections show that this area is a nettle battery. The remaining part of the organ has one important peculiarity: definite longitudinal muscle tracts. The lining endoderm, as in the rest of the body, is made up of elongated epithelium cells, packed with algae, and provided with muscle processes. In Figs. 5 and 6 both endoderm and ectoderm are thrown into folds, because of the contracted condition of the animal. Beneath the nettle battery the endoderm cells are lower than elsewhere, and are not thrown into folds, though in the organ shown in Fig. 5 contraction has drawn them some distance away from the ectoderm. The musculature of the stinging organs appears to be exclusively endodermal and longitudinal. There may be scattered transverse fibres, but I could not observe them. The fibres belonging to the endodermal area just beneath the battery are not gathered into bands, but elsewhere (Fig. 5) there are well marked longitudinal muscle bands, which, however, have not a symmetrical arrangement. The ectoderm of the organ does not differ from the general body ectoderm, except in the region of the nettle battery, which I shall now describe.

The nettle battery, besides the nettle cells in their various stages of development, contains the universally present supporting cells, and a very few gland cells—both the slender, granular gland cell and the swollen mucus cell. The perfect nettle cells are very large, with a closely and regularly coiled filament, which is nearly transparent. I could not detect any superficial

heap of protoplasm or a nucleus. The sectional method is not a very good one for the study of the development of such things as nettle cells, still certain points come out clearly enough, and these indicate that the nettle cells in the actinia battery develop in a different way from that followed by the nettle cells of Siphonophore batteries.

In Siphonophores, according to Bedot (Rec. Zool. Suisse, 1886; Jour. Roy. M. S. 1887), the nematocyst or capsule appears as a vacuole in the mother cell. From the wall of the vacuole grows out a bud, which forms the filament. The transparent substance filling the vacuole condenses round its periphery to form the capsule coat. The formation of a capsule takes place in quite small cells—in sections through a Physalia battery all sizes of capsules are found, from very little ones up to the large and perfect cysts, ready to discharge. Now in the actinia battery nothing of this kind is found. There are no very small cysts which (as in Physalia) increase in size with the growth of the containing cell; and there is no formation in the cyst-producing cell of a vacuole from the wall of which the filament grows out as a bud.

In the actinia the wall of the capsule takes the stain, the contents remain transparent and colorless. Besides the ripe capsules others are found (*a*, Fig. 5), which are smaller and have a concavity on one side, filled up by a heap of protoplasm. These capsules are not found at the surface, as are the ripe ones, but lie lower down, sometimes with their long axes nearly tangential to the surface. They are of graded sizes, but none very small. The contents is finely granular and stains well with haematoxylin. Each capsule contains a number of large granules, which stain very deeply. Other capsules are occasionally found, such as *x*, Fig. 5, the contents of which, though taking a decided tint, is fluid. Such capsules contain one or two short and very irregularly coiled filaments, which, like the granules in *a*, take a deep stain. I regard *x* as an intermediate stage between *a* and the perfect capsule. The granules which have been formed in *a* continue to increase at the expense of the finely granular contents, and fuse into a chain, the contents being by this time fluid. The chain, at first short and stainable, gradually changes its nature, and becomes the closely coiled and transparent filament of the ripe cyst.

3. *Morphology of the Stinging Organs.*

The stinging organs at first sight would seem to be modified tentacles. But two facts negative this hypothesis. In the first place each organ is derived from a chamber, which also gives rise to one of a cycle of tentacles. In the second place the longitudinal muscular system of the tentacles is ectodermal (Fig. 5), while the same system of the stinging organs is endodermal.

Another homology immediately suggests itself which is more satisfactory. In certain actinias, just below the tentacles the lateral body wall forms a circle of little pouches, known as marginal sacs (bourses marginales, randsäckchen). These organs are especially conspicuous in Actinia mesembryanthemum, where they are of a bright blue color, and have attracted the attention of all zoologists who have busied themselves with this anemone. The observers who preceded the Hertwigs were in general agreed in ascribing a sensory function to the marginal sacs. Thus Schneider and Rotteker (Ann. and Mag. of N. H., vol. VII) and Dana (Corals and Coral Islands) regarded them as eyes. Korotneff, on the other hand, showed they were not eyes, but erred himself in concluding they were special touch organs. He describes the ectoderm of the sacs as containing, besides nettle cells, peculiar touch cells.

The Hertwigs (Die Actinien) were not able to study the structure of the marginal sacs in Actinia mes., but carried out their investigation on a closely allied form, Anthea cereus. The pouches turned out to have an ectoderm not essentially different from that of the rest of the body. Its peculiarity lay in the possession of a large number of nettle cells. Marginal sacs, then, are small, spheroidal diverticula which arise from the interseptal spaces just beneath the tentacles, and which possess an ectoderm rich in nettle cells. They serve as weapons.

In the matter of position and essential structure the four stinging organs of Hoplophoria agree with the marginal sacs of Actinia mes. The large size and the high development of the musculature alone mark off the former from the latter, and I regard the two structures as homologous. The ancestral form of Hoplophoria very probably had a circle of small sacs, much as in Actinia mes., just beneath the tentacles. As the habit of living in a hole was acquired, certain of these sacs were transformed

into large and formidable weapons, which were of use in keeping out unwelcome intruders. The remaining sacs were lost.

4. *Systematic Position of Hoplophoria.*

If, now, we come to the question, where does Hoplophoria belong, the answer would seem to be with the Antheadae (Hertwig). The Antheadae are Hexactiniae with long, simple, marginal tentacles, and with the endodermal circular muscle so feebly developed that the oral disk can either be not covered at all or only incompletely covered. Acontia are absent. According to Hertwig, the numerous septa reach for the most part up to the oesophagus, and probably all bear reproductive organs. Marginal sacs are found in certain members of the family (Anthea cereus, Actinia mes.)

Now as respects important qualities, such as the character of the tentacles and the oral circular muscle, and in the absence of acontia, Hoplophoria agrees with the above description of the Antheadae. In certain other peculiarities it differs. The points of difference are as follows: (1) Possession of only four marginal sacs, which are highly developed. (2) Only the six primary pairs of mesenteries reach the oesophagus. (3) Only four mesenteries bear generative organs.

The fact that the undifferentiated homologues of the stinging organs, where they are found, are found in large numbers (as a circle of marginal sacs), leads one to believe that Hoplophoria has been derived from a form with a circle of marginal sacs. The possession of the stinging organs cannot therefore be regarded as of high classificatory value.

As regards the second point, that only the six primary pairs of mesenteries are complete, Hoplophoria is a Sagartid; Hertwig (Challenger Report) regards as one of the distinguishing peculiarities of the Sagartidae, the fact that only the six primary pairs of septa are complete, and at the same time sterile. It is obvious, however, that Hoplophoria is not a Sagartid; in all the *important* qualities above enumerated it differs from this family. The inference is that many actinias may independently acquire the peculiarity of having only the six primary pairs of septa complete. This point cannot, therefore, be of high classificatory value.

The differentiation of four septa as the only fertile septa is odd, but ought not to exclude an animal from a family which it resembles in several important points of structure. The power which each mesentery of an actinia primitively possessed of developing generative organs has afforded (as in other cases of the multiplication of similar parts) an open field for the play of variation and selection. The differentiation of the mesenteries into sterile and fertile has taken place independently in several different ways. In the Sagartidae only the secondary (incomplete) septa are fertile; the primary and complete septa bear no generative organs. In Hoplophoria, on the other hand, four of the primary septa have been singled out as the fertile ones.

The three peculiarities which place Hoplophoria apart from the Antheadae have now, I think, been shown to be of low classificatory value; they are such as have been (2 and 3) independently acquired by several actinias, or (because the modification of certain of a large number of similar structures is so common) may be (1) so acquired. Our anemone is therefore to be regarded as a genus of the Antheadae which has been derived from some such form as Actinia mesembryanthemum. The peculiarities in the possession of which it differs from such a form are adaptations to its habit of living in a hole excavated in coral rock.

Whether there is any relationship between Hoplophoria on the one hand and the Ophiodiscus of Hertwig and the peculiar Lebrunia neglecta on the other, cannot as yet be discussed. Prof. McMurrich (Johns Hopkins Univ. Circ. No. 70) includes Lebrunia in his list of Bahamian actiniae, and a description of this interesting form will no doubt be given in the paper on the actinias of the Bahamas which Prof. McMurrich tells us he is about to publish in the *American Journal of Morphology*.

BALTIMORE, *April* 25, 1889.

DESCRIPTION OF THE FIGURES.

FIG. 1.—Oral view of the living animal, X 20. The part *n. b* of the stinging organ is the battery of nettle cells.

FIG. 2.—Side view of living animal, X 20.

FIG. 3.—Diagram of animal (complete on one side), showing position of stinging organs (*St. o*), arrangement of mesenteries and tentacles (4 cycles, 1, 2, 3, 4), and of generative organs. Pair

of directive mesenteries marked *D* is assumed to be dorsal, opposite pair ventral.

FIG. 4.—Part of a transverse section through the whole column. *St. o* is a stinging organ, *T* is a small tentacle. A pair of mesenteries of each cycle is present—one of the primary pair (1) is fertile. Zeiss, A 4.

FIG. 5.—Transverse section through the middle of a stinging organ. Battery of nettle cells above. To the right is a small tentacle in transverse section. Zeiss, A 4.

FIG. 6.—Longitudinal section through a stinging organ, and the tentacle immediately above it. Zeiss, A 4.

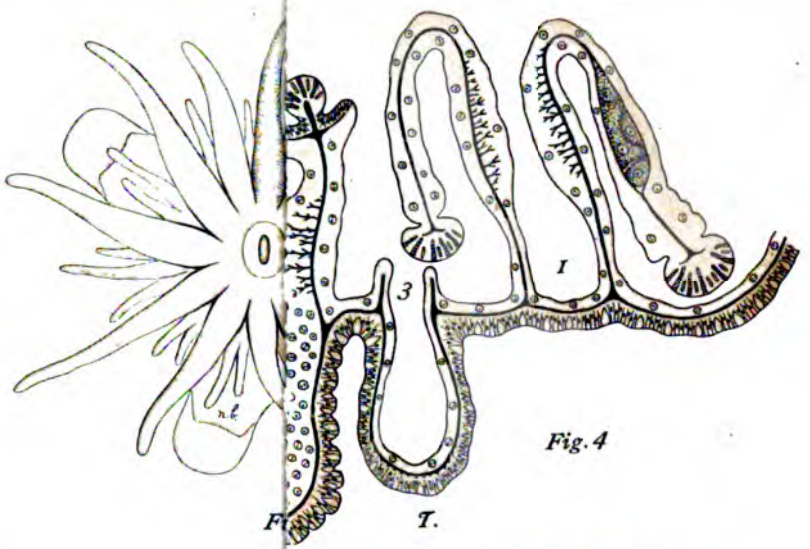

Fig. 4

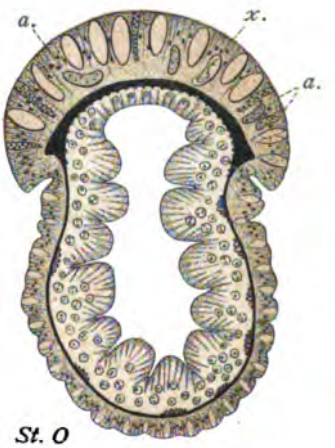

Fig. 5

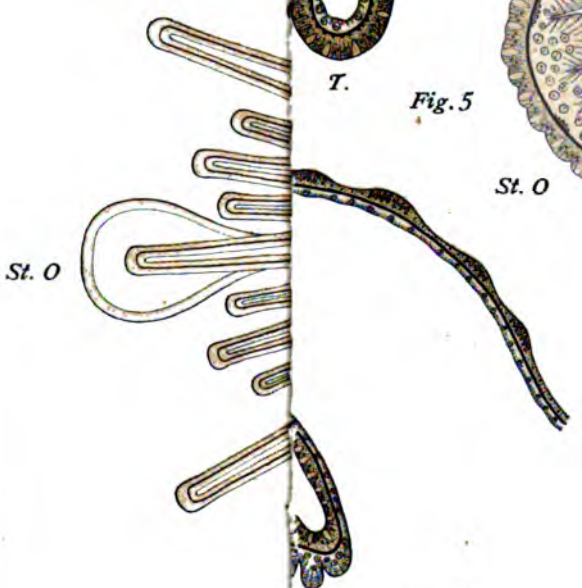

Fig. 6

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NOTES ON THE ANATOMY OF SIPUNCULUS GOULDII POURTALÈS.

By E. A. ANDREWS.

With Plates XLIV, XLV, XLVI, XLVII.

The following observations upon the anatomy of this Sipunculid were made upon material obtained at Wood's Holl, Mass., while I enjoyed, through the kindness of Prof. M. McDonald, the privileges of the laboratory of the United States Commission of Fish and Fisheries.

The original name, as given by the discoverer, Pourtalès (1), has been used in preference to *Phascolosoma Gouldii* of Diesing (2, 3), Baird (4), Keferstein (5, 6), and others, from the adoption of the generic characters as emended by Selenka (7).

The characters of the species are well given by Keferstein and by Selenka. A diagrammatic sketch of the general architecture of this Sipunculid may be here given before speaking of the habits of the animal, closely associated with its peculiar structure.

The smooth, cylindrical body in the expanded condition tapers at each end and measures about 280 mm. in length and 8 mm. in greatest diameter. It may be regarded as made up of two cones applied base to base, the short anterior one having the simple mouth at its apex, surrounded by outgrowths acting as branchiæ; the posterior one ending in a closed point (Fig. 1). Two nephridia (*R N*) open near the base of the posterior cone at the junction of the lateral and ventral aspects, while the median, dorsal anus lies somewhat removed from the base. The anterior cone is only one-fifth to one-fourth of the entire length of the body, and joins the posterior cone without any abrupt demarcation. In contraction the anterior cone is rolled into the posterior one by successive invagination from the mouth at the apex to the base near the nephridial openings. There then remains at the base of the posterior cone a small transverse slit, of crescentric form, convex dorsally. The posterior cone also becomes shorter and thicker and curved more or less, especially at the anterior part, so that the anus is on a convex dorsal line.

The anterior cone was usually spoken of as the proboscis, but is now preferably called the introvert. The tough, muscular body wall surrounds a large, continuous body cavity filled with liquid; in the anterior cone it is not as thick as in the posterior. The digestive tract hangs in a loop from mouth to anus, extending down near to the apex of the posterior cone, and attached anteriorly only by four slender threads, two on the right and two on the left, to the ventral body wall (Fig. 1). The descending loop, from the mouth, and the ascending loop, towards the anus, are connected with one another, and the latter to a muscular membrane (*IM*), about which both loops are spirally wound throughout the major extent of the posterior cone. The nephridia are two dark-colored tubes (*RN*) as long as the anterior cone, hanging freely in the body cavity, and, like the digestive tract, moved back or forth by currents of liquid. The nerve cord (*IN*) along the median, ventral line of the body cavity is attached to the body wall, and like it differs in the two cones: at the apex of the anterior cone it passes around both sides of the digestive tract, to end in a median, dorsal ganglion. The branchiæ (*Br*) all communicate with a tubular sac closely applied to the dorsal surface of the digestive tract, and ending blindly in the anterior part of the posterior cone (Fig. 1, *Bls*). This sac contains a colored, corpusculated blood. The anterior cone is drawn into the posterior one by four retractor muscles (*RDR*, *RVR*), passing from the apex of the former to the body wall in the latter, two shorter ones being attached to the dorsal surface near the base of the posterior cone, two much longer and larger ones to the ventral surface near the nerve cord, one on each side. Along the posterior faces of these last, where attached, and across the median line between the nerve cord and body wall, is a ridge of peritoneal cells, forming the eggs in the female and spermatozoa in the male (*RO*).

This Sipunculus is very abundant in certain small areas of compact, fine sand darkened by organic matter and not quite laid bare at ordinary low tide. In such places, only a few square meters in extent, they pierce the sand in all directions to a depth of more than half a meter, making burrows with persistent lumen running from the surface downward and then laterally, but with no regularity in direction.

Kept in aquaria, the dependence of the animal upon the nature of the sand and its method of locomotion may be readily observed. A vigorous individual buries itself in a few moments, in the following manner: Running out the introvert to nearly its full extent and applying it to the surface of the sand till some spot of less resistance is found, the animal still farther expands the introvert so that it penetrates the sand, provided this is not too dense and firm, for then the body is merely shoved backward. When the introvert is inserted, the contraction of the longitudinal muscles of the body wall brings the whole body forward somewhat, in case the introvert is fixed in the sand. In case soft ooze was present, this fixation did not take place, and the introvert was merely pulled out again, but when the sand was of the right consistency the introvert was fixed by becoming much swollen at the tip and then constricted just posterior to this swollen area. This bulb-like area exerts lateral pressure on the sand, as could be seen by movements of the grains. The swelling of the anterior end of the introvert is brought about by the body wall contracting elsewhere and forcing in liquid to distend that end. Owing to the curved form assumed by the body in the normal contracted state when first removed from its burrow, the entrance of the introvert may often be nearly vertical, and hence the entire body is soon raised nearly upright in the water above the sand. If the body has thus been warped forward sufficiently to become somewhat fixed in the sand, the introvert is rolled in and again thrust forward from this new point of resistance, and so on till the animal is entirely buried. This locomotion increases in speed as the creature becomes more completely surrounded by sand, and is the only means of moving from place to place.

On a smooth surface or on one not presenting the right degree of resistance the Sipunculus does not change its position, but remains till death finally occurs, rolling its introvert in and out and contracting its body wall to no purpose.

The essential factors in the mechanism bringing about this *hydrostatic* locomotion are an elongated, contractile sac filled with liquid, and some means of definitely co-ordinating the contractions of the sac.

In natural environment the animals are found with sometimes

one, sometimes the other end nearer the surface of the sand: in the aquaria the same was observed, but when the water became stagnant and impure the anterior end with expanded branchiæ was often protruded somewhat above the surface of the sand.

Body Wall.

In this we recognize three chief concentric regions, the cuticle, the epidermis, and the muscle layers with their peritoneal covering (Fig. 3).

Cuticle (*Ct*).—This forms a thick, tough covering to the body, presenting the physical appearance of chitin, but dissolving in boiling KOH, and probably not identical with the chitin of the crustacea. It is easily thrown into wrinkles and folds, and has much the same structure as in the Chaetopoda. A homogeneous matrix is penetrated by innumerable, very delicate fibrils that are arranged in an external and a more internal system, the two extending nearly at right angles to one another. On the worn outer surface the ends of such fibrils may project somewhat, as short irregular lines.

On tearing off the cuticle it is found divided by folds or creases into quadrate areas about 100 x 150μ, each with a central conical depression 90μ wide, opening at its apex onto the external surface of the body through the thin part of the cuticle present there. These conical pits in the thick cuticle correspond to elevations of the epidermis upon which the cuticle is moulded, while the minute openings at the apex are places in which the epidermis comes to the surface with no cuticular covering.

Epidermis (*Ep*).—This may be regarded as a single-layered epithelium, specially modified to form a peculiar organ in each of the above pits in the cuticle, and having the inner ends of its cells imbedded in a nearly homogeneous matrix that intervenes between them and the muscles of the body wall, and which may be demonstrated as forming irregular ridges and elevations when the epidermis is macerated off. The cells of this single epidermal layer are somewhat pyramidal, their external bases forming a close mosaic of polygonal outlines as seen from the outer surface, while their internal ends are somewhat separated and pointed. The large nuclei are nearer the external than the internal ends, and the cell protoplasm is full of yellow pigment

granules. When the epidermis is thrown into folds or stretched, the shape of the epidermal cells apparently also changes.

Epidermal Organs.—These swollen, conical masses of epidermal cells rising above the muscular layers to occupy the above-mentioned excavations in the cuticle are very thickly and irregularly scattered all over the body (Fig. 2). At the anterior and posterior ends of the animal these organs are small and of uniform size, but in the middle region they vary from 30μ to 80μ. Here they cause but little elevation of the cuticle externally, while in the former regions they project as evident papillæ above the general surface of the body. Where the body wall is contracted each organ is circular in outline; where expanded, drawn out transversely into an elliptical outline.

In structure these organs present three different forms:

1. The largest organs are made up of many, perhaps twenty, granular, elongated cells closely crowded together to form a somewhat pear-shaped mass, with the narrower part passing externally into the ordinary epidermis which lines the conical excavation in the cuticle (Fig. 3, *Ep Gl'*). Each cell has a small nucleus in its lower portion and an elongated vacuole with sharp, definite walls in its outer portion, extending throughout about one-half the length of the cell (Fig. 7, *A*). The contents of these vacuoles or sacs is homogeneous, watery, and stains violet with methyl green when the protoplasm stains blue. The cells are so crowded that the superficial ones are bent over the central ones, bringing all their external ends to one point where ends a minute tubule, apparently an ingrowth of the cuticle and stiff. The sharp wall of the vacuoles ends near this point, and it appears as if a communication might here take place from the vacuole to the tubule or duct. The inner ends of the cells have delicate processes (*Pr*) attached, which, as the rounded inner end of the organ is connected with the nerve plexus of the body wall (Fig. 3), may be regarded as nerve processes.

2. Among the smaller organs are many (Fig. 3, *Ep Gl*) made up each of a few—less than ten—large pear-shaped, sac-like cells filled with large refractive protoplasmic granules so closely packed as to be angular. The nuclei are in the lower rounded ends of these cells, while the outer ends taper to slender processes extending out to the surface of the body (Fig. 7, *C*).

Both the above kinds of organs may be regarded as glands, though no conspicuous mucous or other secretion, odor, taste or reaction to litmus paper could be perceived on the surface of the animal. In teased preparations small collections of granular matter were, however, sometimes seen at the external openings of these bodies.

They are all imbedded in a connective tissue jelly (Fig. 8, *Sep*), and have delicate coats of connective tissue with some indications of nuclei. This connective tissue appears to extend in between the large cells of the second sort of gland.

3. At the anterior and posterior extremities of the body some of the organs in the papilla-like elevations have a different, non-glandular structure. Each is a collection of numerous elongated slender cells with large nuclei; the peripheral cells of the elongated mass being more or less flattened to serve as an envelope to the slender central cells (Fig. 7, *B*, *b*). These latter (*a*) end externally in slender processes extending towards or probably to the outer surface of the body, and internally in very delicate processes, probably nervous.

The entire organ may be regarded from its position, connection with nerve plexus and structure, as a sense-organ.

An examination of the epidermis in regenerating posterior tips of mutilated animals leads one to infer that all three kinds of epidermal organs arise merely as solid, plug-like ingrowths of the undifferentiated epidermis.

Muscular Layers.—The main thickness of the body wall is made up of two systems of muscle fibers, a thinner outer, transverse series (Fig. 8, *C M*) and a thicker inner longitudinal series (*L M*). In the extreme posterior tip of the body and in the introvert the muscular layers are reduced in thickness; the longitudinal system especially in the latter region, where it forms a delicate continuous sheet, though elsewhere divided into thirty or forty longitudinal ridges or bands separated more or less completely by deep longitudinal grooves (Fig. 1). These grooves are, however, crossed by frequent oblique bundles of fibers passing from one ridge to another.

The muscle fibers are very long and pointed at each end. Each has a granular nucleus-like mass of protoplasm imbedded in it midway between the ends, forming an eccentric axis at that point.

Some cases were observed in which the fiber bifurcated at the center, each of the resulting three prongs containing some of this granular material.

The rest of the fiber is homogeneous and highly refracting, but may be resolved into many longitudinal fibrillæ by maceration in chromic acid.

The fibers in the external part of each system are smaller and more crowded than in the internal portion, and conspicuously arranged in radiating lines, separated by some connective tissue matrix; when seen in mass they have a beautiful, silky lustre.

Internally the peritoneum (*P*) forms a delicate layer, covering the longitudinal muscles and extending into the longitudinal grooves between the muscular ridges.

Peripheral Nerves.—The body wall is supplied with a very rich meshwork of nerves that are arranged in three concentric cylinders with radiating connections (Fig. 33). Between the circular and longitudinal muscles there passes around the body a system of large nerve rings, completed ventrally by their origin from the nerve cord and also by smaller nerves between the above muscle-layers (Fig. 22, *Cn*). On the peritoneal surface of the longitudinal muscles is a second system of nerves, forming there a delicate reticulum (Fig. 9). On the external surface of the circular muscles, next to the epidermis, is a very intricate reticulum of nerves with larger longitudinal trunks (Fig. 26).

The last-mentioned reticulum gives off numerous radiating nerves inward to the circular muscles, and these in turn have branches passing transversely among and along the muscle fibers.

The first-mentioned system of circular nerves gives off numerous radiating branches, passing inward in the longitudinal muscles to connect with the reticulum on the peritoneal surface of these muscles, and giving off, on the way, numerous longitudinal branches among and along the fibers.

With reference to the muscles there are thus two chief sets of nerves: the sub-epidermal longitudinal branches of a reticulum; the inter-muscular series of circular nerves connected with the ventral nerve cord. Each set lies external to and at right angles with the fibers that it ultimately supplies by means of radiating branches and respectively circular and longitudinal sub-branches.

These two sets of nerves are in turn connected with each other

by radiating branches passing outward from the circular nerve trunks through the transverse muscles to unite with the main longitudinal nerves of the sub-epidermal reticulum. The entire muscular body wall is thus penetrated by a complex plexus of nerves connected ventrally with the nerve cord, as will be described later (Fig. 33, 22).

The sub-epidermal plexus sends nerves to the epidermal organs.

The inter-muscular plexus innervates the muscles: fine branches passing along the fibers and finally ending in minute swellings, after branching somewhat on the sides of the individual fibers. These minute terminal swellings were seen only in gold preparations and appear to be motor plates, though no structure was made out.

Body Cavity.

This large space has projecting into it the digestive tract, the nephridia, the nerve cord and the reproductive organs (Fig. 1). It is lined throughout by a thin membrane of flat cells with irregular outlines, as shown by silver nitrate (Fig. 8); these, perhaps, changing in complexity in various conditions of tension of the cells in movements of body wall, etc., being often extremely sinuous and interdigitated. The nuclei of these cells project slightly into the body cavity above the general level of the thin cell body. Some of the nuclei are associated with granular protoplasm bearing tufts of numerous long cilia, these ciliated cells being irregularly scattered all over the body walls, nephridia, digestive tract and retractor muscles, but not present, as far as could be determined, upon the nerve cord and its branches, nor upon the connective tissue strands supporting the intestine. Special modifications of these peritoneal cells will be described in connection with the "pseudostomata" of the digestive tract.

Contents of Body Cavity.—The body cavity is filled with liquid, in which are various solid elements, in addition to the chloragogue cells that project from the peritoneum.

These latter form an easily removable layer of delicate cells (Fig. 23, 21, *Ch c*), more or less completely clothing the intestine and projecting from the peritoneal layer into the body cavity. They vary much in form, are often club-shaped, about $36\mu \times 6\mu$,

with nucleus 2μ, and often completely filled with bright yellow spherules less than 1μ in diameter. Similar cells occur scattered on the connective tissue strands supporting the intestine; while on the nerve cord there are scattered yellow granules and aggregates of such about the nuclei of a few scattered, granular cells that appear much like the ciliated peritoneal cells, and differ from true chloragogue cells in containing the yellow pigment in the form of much more minute granules.

There thus seems to be a correlation between presence of yellow pigment cells and absence of ciliated cells on the peritoneum.

The solid elements freely floating in the liquid of the body cavity include two sorts of corpuscles, male or female cells, and a peculiar element of unknown significance.

The most numerous corpuscles give the pink color to the liquid of the body cavity (Fig. 15, *C*). Each is a disk, slightly biconvex, nearly circular, measuring 6 to 24μ in diameter and about 3μ in thickness, transparent, refracting, and homogeneous when fresh, slightly yellow seen singly, and apparently of the same consistency as a vertebrate red corpuscle. Like these they are highly elastic, returning to the average shape after being drawn out, by currents, etc., into greatly elongated forms. Reagents reveal a granular, eccentric nucleus, while the surface may become crenated as in mammalian red corpuscles. The number of these corpuscles is indicated by the average of determinations by the hæmacytometer from four individuals. This was, in round numbers, 90,000 colored corpuscles per cubic millimeter, or, as there is about one cubic centimeter of body cavity liquid in an average individual, 90,000,000 colored corpuscles for the entire animal.

The second sort of corpuscle (*C''*) is the granular, amoeboid, white corpuscle, which occurs in much less numbers. Estimated as above there are only 3000 per cubic millimeter; but this is probably too small an estimate, from the fact that these cells adhere to the glass and also in part break down rapidly. They vary in size through about the same limits as the colored corpuscles, but are usually smaller, granular, nucleated cells with stiff, clear pseudopodia.

The less numerous larger ones seem to form a distinct variety full of definite, rounded or elongated granules (*C'*).

The third solid element (*G c*) may be called the "giant corpuscle," as it measures 32 to 123μ in diameter, with a thickness of 42μ in the largest. They have the same shape as the colored corpuscle, but are colorless and enveloped in a membrane-like outer coat that contains one to nine nuclei with innumerable radiating and anastomosing lines, which may be protoplasmic, though appearing more like ridges in the membrane. When fresh these corpuscles are very transparent and have much the same consistence as the colored corpuscles.

These are found in both males and females, and are less numerous than the amoeboid corpuscles. That they have some relation to the colored corpuscles is suggested by their similar consistence and shape. In the female, eggs in various stages of growth are found floating in the body cavity, often in great quantities; though in one estimate less numerous than white corpuscles, they may exceed that number, I judge. The smallest measure 24μ, with a nucleus 12μ, and are spheres with definite outline and granular protoplasm containing some of the yellowish granules subsequently so characteristic of the egg.

Floating thus free in body cavity liquid the egg grows to be 50μ before the nucleus is obscured by the accumulation of granules. Later an almost invisible gelatinous case is formed around the opaque, granular, brownish egg; one measuring 100μ has a case 6μ thick. The gelatinous envelope increases in thickness in the same ratio as does the egg. Eggs that are apparently ripe (Fig. 14, *O*) have a diameter of nearly 200μ, and are encased in a vitelline membrane (*z*) 3μ thick, penetrated by radial pores, through which delicate pseudopodia-like processes pass out into the outer gelatinous envelope (*P*), which may be 12μ thick, and seems to aid in the adhesion of the eggs to foreign objects when removed from the body.

In the male the body cavity contains innumerable spermatozoa in process of formation or complete, and sometimes moving before exposure to sea water (Fig. 14, *S*). An attempt to estimate them by the hæmacytometer indicated that one cubic millimeter may contain 1,700,000 perfect spermatozoa. As in the preceding cases, these estimates are subject to very great error; but the actual number of spermatozoa present is sufficient to make the entire body cavity liquid turbid and milky white. Each spermatozoon has a top-shaped head 4 x 2μ, with tail 60μ.

The liquid part of the contents of the body cavity is clear, colorless, somewhat heavier than sea water, having a strongly salt taste and neutral reaction, and producing a heavy precipitate with silver nitrate.

When removed from the body it clots very quickly. The clot is a gelatinous one, formed independently of any colored corpuscles, and contains fine fibrils having the appearance of fibrin. When the clot is formed under the cover-slip, the larger, granular, amoeboid corpuscles spread out pseudopodia that unite with those of other corpuscles, while the smaller amoeboid remain in normal condition. Soon the larger corpuscles seem to break down and to lose their peculiar granules. The fibrin filaments often radiate from clear masses, appearing as if formed from the broken-down larger sort of amoeboid corpuscle.

Branchiæ and Vascular System.

The branchiæ, or tentacles (Fig. 11), form a circular group of processes around the mouth, projecting forwards and outwards when expanded, so as to present a similarity to the tentacles of an actinian; and closely crowded in a circular mass on the inside of the introvert when it is withdrawn, then appearing somewhat in clusters at the ends of longitudinal ridges passing into the mouth.

When the mouth is closed and the branchiæ expanded, the body wall passes over into the digestive tract by a disk-like area or evaginated lip, on which fleshy ridges radiate out to the branchiæ about the edge of this disk-like area. Of these ridges radiating out from the closed, terminal mouth there are two chief ones, dorsal and ventral on the median line, two ventro-lateral of less size, two dorso-lateral yet smaller, and interpolated between these six two sets of more peripheral radiating elevations; the chief set counting six ridges, the subordinate set having a greater number arranged between the others, so that there would be twelve if this set were complete all around the circle (Fig. 12). The ridges end peripherally in a continuous circular membrane bearing the branchiæ as outgrowths of its free, anterior margin and extending centrally as the outer part of each ridge, which thus bears a double row of branchiæ peripherally, each row being continuous with the adjacent row of the

next ridge. The branchiæ thus appear arranged in clusters corresponding to the chief ridges (Fig. 11). Their arrangement may be better expressed by supposing them to be outgrowths of a circular membrane that has been tucked in towards the central mouth-opening along certain radiating lines, where it forms ridges on the everted lip, some of which come nearer to the center and bear more branchiæ, others, but slightly infolded from the periphery, bear few branchiæ (Fig. 12).

The branchiæ are symmetrically arranged; a median dorsal free part of the membrane bearing no branchiæ is distinguished from the median ventral part: the right and the left series are in the main alike.

The entire number of branchiæ may be as great as 200, nearly equally distributed right and left. Of these the longest are on the ridges and nearest the mouth, the shortest are peripheral, on the membrane where joining adjacent ridges.

In the youngest individual obtained, which measures 13 mm. in entire length and 1½ mm. in greatest diameter, the branchiæ are arranged in a simple row arising from a circular membrane that presents a marked dorsal median region free from branchiæ and a similar median ventral one. On each side there are only twelve short branchiæ, twenty-four in all.

Each branchia or branchial filament is a blunt, elongated process, somewhat flattened, hollowed out by a longitudinal ciliated groove along its inner, oral, side and convex on the opposite side, so as to present a triangular transverse section. During life the branchiæ vary much in size and form as well as position of tip, being muscular and highly contractile.

A rapid current of blood passes up to the tip of each through two lateral channels, and there turns back to the base of branchia through a single median channel along the convex face. Besides the main communication between these three channels at the tip there are numerous irregular, lateral openings from the median to each lateral channel, through which some blood passes into the median channel. In each lateral channel numerous transverse, incomplete partitions project from the grooved face of the branchia towards the convex face and form pockets or chambers, in which some of the blood circulates in whirlpools before passing along towards the tip with the main stream.

The structure of the branchiæ is as follows: The thin cuticle covers a single layer of epithelial cells, which are ciliated only on the concave oral surface and are there also elongated, while over the convex, aboral surface they are not ciliated and nearly cubical towards the base of the branchia, but passing over into the columnar cells towards the tip. The elongated cells of the ciliated surface bear, each, large numbers of cilia; some of these cells, however, are unicellular glands and irregularly scattered amongst the ciliated cells. The main mass of the branchia is made up by the three large blood-vessels, lined by a membrane with prominent nuclei and occasional cells, with tufts of cilia. This membrane closely resembles the peritoneum; its cilia cause the violent currents of blood corpuscles in definite circulatory order. Between these vessels and the epithelium there is a layer of connective tissue having a matrix filled with fine fibers, circular, longitudinal and radial, and many cells, some of which have fine processes. These fibers appear to act as muscles, as the branchiæ are highly contractile.

Branching nerves pass through the same connective tissue, springing chiefly from a large longitudinal median trunk close to the epithelium at the bottom of the oral furrow.

When the branchiæ are fully expanded they stand in radiating rows, so that the concave side is not necessarily turned towards the mouth, but opens at its base into a radial furrow, between successive infoldings of the circumoral membrane (Fig. 11), and its cilia are thus continuous with the ciliated surface of the evaginated lip or oral disk.

The *oral* or concave surface of the branchiæ is thus only a continuation of the lining of the mouth, and differs much from the convex, outer surface, which in turn is a continuation of the outer layer of the body wall.

Vascular System.—The three longitudinal vessels in each branchial filament open into a complex network of vessels in the oral disk, which network is continued around the epithelium of the digestive tract, imbedded in connective tissue, as far as the pharynx extends or to the commencement of the œsophagus. Just anterior to the nerve commissures, however, this network opens into a larger, circular vessel surrounding the pharynx and ending dorsally in a median enlargement just ventral to the brain (Fig. 32, *Bls*).

From this enlarged blood space a median dorsal vessel passes back along the pharynx and the œsophagus to its posterior end. This latter portion of the dorsal vessel forms the peculiar blood sac attached along the dorsal surface of the œsophagus but otherwise surrounded by the body cavity fluid (Fig. 1). When the œsophagus is contracted and empty this blood sac may have an equal diameter and is conspicuous from its peculiar red color, due to the great numbers of red corpuscles contained in it. Externally the sac is covered by the peritoneum with ciliated cells and chloragogue cells; internally it has the same nucleated membrane as the vessels in the branchiæ, and also scattered tufts of cilia which keep the blood corpuscles in constant motion. Between these two layers is a very thin layer of connective tissue, in which are many transverse or circular fibers arising from the ventral side, where the connective tissue is continuous with that in the wall of the œsophagus. As the peritoneum continues over both sac and œsophagus also, the sac may be regarded as a greatly enlarged vessel hollowed out in the connective tissue part of the wall of the œsophagus as the other vessels are in the wall of the pharynx and in the branchiæ.

The circular fibers of the sac are contractile and cause irregular constriction of the walls, so that the sac presents rounded lateral pouches, which are not permanent.

The whole sac is somewhat contractile, and pulsates even after the blood is removed. At the posterior end the sac presents a blunt, sudden rounded termination (Fig. 1, *Bls*), with no connection with the body cavity that could be found.

The contents of all the above vessels is a liquid that coagulates rapidly and contains three kinds of corpuscles like those in the body cavity; but it has none of the giant cells. No spermatozoa nor egg were found in these vessels, or other evidence of any connection with the body cavity.

Moreover, the red corpuscles are smaller than the average of those in the body cavity (Fig. 16), 10 to 15μ wide and 4μ thick, and much more numerous in a given volume, about seven times, as indicated by the hæmacytometer. The nuclei are generally larger relatively, and often absolutely, than in the red corpuscles of the body cavity.

The Digestive Tract.

This forms a tube of nearly uniform diameter from mouth to anus, prolonged posterior to the latter opening as a loop that reaches nearly to the posterior tip of the body, and is spirally wound from left to right about forty times.

Six regions may be distinguished: (1) Pharynx, (2) œsophagus, (3) stomach, (4) descending intestine, (5) ascending intestine, (6) rectum.

The pharynx is the short buccal cavity or part into which the branchiæ are withdrawn, and suddenly diminishes in caliber on becoming the œsophagus. The œsophagus is accompanied by the dorsal blood sac, and extends back beyond the anus, its anterior part being, like the pharynx, closely surrounded by the four fused retractors, its middle part attached to the separated retractors by four series of radiating connective tissue strands, and its posterior and much longest part being entirely free in the body cavity, except for a single connective tissue thread (Fig. 1).

The stomach is a short part making only two turns around the ascending intestine, and marked by four longitudinal reddish bands.

The descending intestine reaches from the stomach, about the region of attachment of the ventral retractors, to the commencement of the ascending intestine at the extreme posterior end of the intestinal loop. It is closely attached to the ascending intestine, about which it winds, by numerous connective tissue strands (which also are found on the stomach and posterior end of the œsophagus, and are there larger and more definite in arrangement).

The ascending intestine is characterized by a longitudinal ciliated groove and by the attachment of a mesentery along its ventral aspect.

The rectum is nearly as short as the stomach, is marked off from the intestine proper by the cessation of the ciliated groove, though the mesentery continues along the rectum to be attached to the body wall posterior to the anus.

The whole digestive tract is freely hung in the body cavity, except for the anterior attachment of the œsophagus to the retractor muscles, of the rectum to the body wall by radiating strands of connective tissue at the anus, and for the presence of

four long, elastic, connective tissue filaments proceeding from the ventral body wall to the œsophagus and ascending intestine, and limiting somewhat the amount of motion of the entire digestive tract back and forth in the body cavity.

These four filaments arise between the anterior and posterior retractors, two on the right of the ventral mid-line, two on the left. The anterior left one is attached to the œsophagus. The other three are attached to the ascending intestine between the rectum and the region connected with the stomach.

The structure of the digestive tract is in general as follows (Fig. 13, 21): A single layer of columnar epithelium (*e*), each cell with a tuft of cilia, forms the lining, while the peritoneum clothes the tube on the outside. The peritoneum has the usual flat, polygonal cells, with occasionally tufts of very long active cilia, and is covered more or less perfectly by chloragogue cells (*Ch c*), which give a yellowish color to the entire digestive tract, modified somewhat by the peculiar red radial fibers present in certain regions.

Between the above two layers is a mass of connective tissue, making up the bulk of the wall of the tract. The matrix appears quite soft and yielding, so that it is expanded into a thin film when the tract is distended. In the matrix are numerous fibers, fibrils and connective tissue cells, besides a rich plexus of nerves (Fig. 19) having the main branches longitudinal. The usual arrangement of the fine fibers is in an outer longitudinal and an inner longitudinal irregular layer (Fig. 21, *f'*) close to the peritoneum and epithelium respectively. Between the latter layers is a well-developed circular layer of larger fibers (Fig. 21, *f*). The connective tissue internal to the circular fiber layer seems to differ in the consistency of its matrix from that outside, in some regions, and contains the above nerve plexus (*n*), lying in part close to the epithelium, and may also contain peculiar red, radiating fibers (*R f*).

The intestine is usually greatly distended throughout most of its extent by masses of coarse sand, but often the posterior bend is full of liquid and somewhat translucent.

Pharynx.—As already mentioned, this is the anterior region of the digestive tract containing the branchiæ when not expanded. It is lined by a continuation of the ciliated epithelium of the

oral disk, or the evaginated part of the pharynx, and contains a rich meshwork of blood-vessels in the connective tissue that intervenes between this epithelium and the anterior terminations of the retractor muscles. Where the pharynx passes posteriorly into the œsophagus there is often an abrupt ventral bend and a diminution in caliber, owing to the contraction of the circular muscle fibers of the œsophagus.

Œsophagus.—As just mentioned, the circular fibers are anteriorly very well developed, and appear to form a sphincter; posteriorly they become less and less prominent near the stomach. The lining of the œsophagus is thrown into numerous longitudinal ridges, which are reduced to four near the stomach.

The nerve plexus in the œsophagus is very easily demonstrated, since the connective tissue mass is simple and free from radiating fibers.

That the dorsal blood sac forms a hollowing out in the connective tissue mass of the wall of the œsophagus—protruding, to be sure, much beyond the cylindrical contours of the digestive tract—has already been noticed in describing the vascular system.

Stomach.—Four conspicuous longitudinal red or pink bands along this region might at first suggest continuations of the dorsal blood sac, but are found to be due to the presence of four great longitudinal ridges (Fig. 13) projecting into the lumen of the tract, and owing their red color to the peculiar radiating fibers that here make up the major part of the wall of the tract and form these ridges.

These fibers appear as soft, translucent, reddish, jelly-like cylinders, when alive, having a diameter of 10 to 16μ and a length of more than 80μ (Fig. 10). The color is the same peculiar pink that the common corpuscles of the blood and body cavity liquid possess.

This mass of radiating fibers being interpolated between the epithelium and the circular muscles, the latter are thrust out near to the peritoneum and become little marked. The main part of the wall is thus made up of four ridges of peculiar radiating fibers, the disposition of which suggests that this region may be a sucking stomach!

Descending Intestine.—The same radial, red fibers continue as longitudinal bands down the intestine and also up the ascending limb, but are not as conspicuous nor arranged in limited definite ridges. The epithelial cells are filled with conspicuous granules, except at the extreme outer end, where clear protoplasm supports numerous long cilia.

In the anterior part the ridges projecting into the intestine have longitudinal rows of ciliated pits (Fig. 29), often 20μ deep, lined by smaller, more slender cylindrical cells which are also ciliated and appear yellowish. The action of these cilia is to produce a spiral current in the fluid of the intestine, so that (in teased preparations) granules from the cells of the pit are brought out in a revolving mucilaginous material into the lumen of the intestine.

In the much longer posterior region of this descending limb these pits are also present along longitudinal ridges, but there are in addition larger pits (Fig. 17), which push out the walls of the intestine so as to project 100μ or so beyond the general contour as numerous minute diverticula.

The epithelial cells in this region are elongated, full of conspicuous granules, and readily lose their cilia, appearing then to give off vesicular portions into the lumen of the intestine.

Ascending Intestine.—The diverticula continue a short distance around the bend on this part of the intestine, but are absent throughout much the greater part of its extent. Three structures may be described in connection with this intestine: the mesentery, the ciliated groove, and the pseudostomata.

The mesentery (Fig. 4, 18): this forms a thin membrane of connective tissue containing longitudinal muscles (*Mf*) (hence the whole has been called the spindle muscle), amongst which is a plexus of nerve fibers (*n*). The mesentery is covered by the peritoneum continued over the intestine and may be regarded as a specialized part of the intestinal wall, separated to such an extent that the contraction of the muscles along its free edge produces a spiral coiling of the ascending limb (Fig. 18)—accompanied by the descending limb, since the two are united by transverse connective tissue bands.

The line along which the mesentery arises, when traced anteriorly upon the rectum, is found to terminate on the posterior

face by breaking up into radial strands uniting with the body wall. As the intestine is twisted, as well as spirally wound, the line of attachment at the bend is not posterior, but anterior; hence the mesentery is attached along the concave side of the bend and extends a short distance upon the descending intestine on the aspect turned toward the ascending limb. If we imagine the intestine to pass straight forward from anus to mouth, the mesenterial membrane would be attached along the ventral or neural aspect!

The ciliated groove (Fig. 18, *C G*) forms a conspicuous character of this region, appearing as a longitudinal red line with a lighter center. The red is due, as in the stomach, to the presence of radiating fibers in the wall of the intestine, which here form two parallel ridges projecting into the lumen and embracing between them the ciliated groove. By the contractions of these ridges the groove may be made very shallow (Fig. 24), but is always recognizable: in it a current of liquid passes, from the action of cilia and possibly also of the radiating fibers, towards the anus during life, and at first might be mistaken for a blood current.

The groove passes to one side of the line of attachment of the mesentery, but not far from it (Fig. 18). At the bend it is continued in the descending intestine for a short distance; anterior to this end a line of ciliated pits continues the line of direction of the groove some distance further. The other termination of the groove is at the rectal end of the intestine, where there is formed a diverticulum (Fig. 1, *R C*) into which the groove opens, or one might say the end of the groove is deeper, swollen, and projects from the wall of the intestine as a short diverticulum.

The size of the diverticulum varies as does its shape; it has a length about equal to the diameter of the intestine when the latter is entirely empty, or about .8 mm., and a width of .3 mm.

The ridges bounding the groove may be applied to one another in such a way as to shut off the bottom of the groove from the lumen of the intestine (Fig. 25). This closed portion is lined by cells markedly different from those over the ridges. Their free ends are rounded, separated, not ciliated, and seem to sometimes break off vesicles or fragments which accumulate as granular coagulations in the tube. The same cells are found continued

into the diverticulum. No cilia were seen; they may have been destroyed in the preparation, in some cases at least; yet their absence seems normal.

The pseudostomata (Fig. 18, *Ps*; 21, *Psc*) form conspicuous objects over the outer surface of the ascending intestine, appearing when alive as minute ciliated funnels imbedded amongst the chloragogue cells and suggesting openings into the digestive tract from the body cavity. Careful examination, with the aid of serial sections, failed to demonstrate any internal opening, however, and led to the conclusion that each "pseudostomum" (Fig. 23, 21) is merely a depression of the peritoneum, forming a blind funnel, partly filled by chloragogue cells and guarded along its protuberant lip by a peculiar ciliated cell (*Ps c*).

Each organ is a convex projection, nearly the quarter of a sphere, covered on its convex face by chloragogue cells (*Ch c*) and hollowed out from the abrupt radial, nearly vertical plane by a funnel-shaped depression which penetrates somewhat into the connective tissue of the intestinal wall. This depression is more or less completely filled up by a large chloragogue cell (*G*) like those covering the convex side of the organ and surrounding it on all sides. The projecting rim of the organ, the free edge of the convex surface where falling off into the depressed funnel, is formed by a large ciliated cell (*Ps c*) which has the form of a spindle bent into a crescent, of which the two horns pass along the sides of the funnel opening to be continuous with the peritoneum under the chloragogue cells, while the middle convex part forms the most prominent portion of the whole organ and contains a large granular nucleus.

This cell may be regarded as a special modification of the ordinary ciliated cells of the peritoneum, curved in connection with the funnel-like depression of the peritoneum. The flat peritoneal cells are shown by silver stain (Fig. 8) to continue down into the funnel as well as over the convex portion of the pseudostomum.

These pseudostomata are very thickly set all over the wall of the ascending intestine from a point near the bend, where the diverticula cease to occur, to the rectal end of the intestine, where they are most numerous and cease rather abruptly. At this point they extend on the side bearing the ciliated groove

quite as far as the origin of the terminal diverticulum, while on the opposite face of the intestine they cease sooner. Occasionally a pseudostomum is found upon the mesentery, some distance from the proper wall of the intestine!

There is no apparent regularity in the arrangement of these organs or agreement in the direction in which the openings of the funnels face.

Indications as to what their use may be seem to be afforded by the fact that when carmine is injected into the body cavity it appears in large granules in the chloragogue cells, especially in those that lie protected inside the funnels, so that the pseudostomata then appear as red spots over the surface of the intestine. The specialized, curved ciliated cells would thus appear to bring solid particles from the body cavity fluid into contact with the chloragogue cells lying in the funnels. The ultimate fate of these particles was not determined.

The Rectum.—Like the œsophagus, this region is lined by simple ciliated cells and thrown into numerous longitudinal ridges. Externally there are no pseudostomata, and the continued mesentery after passing close to one side of the terminal diverticulum of the ascending intestine buries itself within the posterior wall of the rectum, passing as a ridge along the rectum, ultimately divaricates as right and left membranous strands radiating out from the walls of the rectum to the body wall.

The muscles of the mesentery form the main mass of the above ridge, and then pass as a right and left portion out in the membrane to the body wall, piercing the longitudinal system to become continuous with the circular muscles of the body wall.

At the anus these circular muscles continue along the rectum as a much thinner layer of circular fibers around the digestive tract, gradually becoming less conspicuous towards the posterior part of the rectum. The terminal portion of the rectum may be everted, when its longitudinal ridges appear on the surface of the body as radiating lines around the anus, or the body wall may be rolled in for a short distance so that the anus is withdrawn from the surface.

When the mesentery comes to fuse with the walls of the rectum its large longitudinal nerve is seen to leave the center of the muscle and to join the plexus of the wall of the digestive

tract. In fact, the nerves of the mesenteric muscle are to be regarded as merely an isolated part of this plexus that is removed from the immediate wall of the tract.

This plexus becomes continuous with the plexus of the body wall about the anus, as was demonstrated by serial sections.

The Nephridia.

Each nephridium (Fig. 1, *R N*) hangs entirely free into the body cavity from its attachment to the body wall at the external opening. The internal opening is also at this same place, so that the free end, generally directed posteriorly, is entirely closed. The organ is a cylindrical tube, of various shades of brown or yellow color, capable of being drawn out to great length and highly elastic. Near the body wall the tube is swollen slightly for less than one-eighth of its length, and obscurely marked off by longitudinal grooves into an anterior lighter-colored and a posterior darker portion, gradually passing into the common more slender tube posteriorly.

This external division corresponds to the division of the tube internally by a longitudinal partition into an anterior (Fig. 5, *In*) and a posterior portion (*E X*), both communicating with the common posterior tube at the posterior end of the partition: the posterior one opening to the exterior (*N M*), the anterior one to the body cavity (*N P*) close to the body wall. Both portions of the tube are constricted close to the body wall, and there are colorless. The anterior section is firmly attached posteriorly and by diverging membrane-like processes laterally to the body wall, while anteriorly it sends out a semicircular flap parallel to the body wall. The slit left between this flap and the body wall is the nephrostome (*N P*) and leads into the anterior part of the tube, which at the posterior end of the septum communicates with the blind posterior continuation of the nephridium as well as with the posterior portion leading to the external opening in the body wall (*N M*).

Externally the nephridium (Fig. 20) is covered by the peritoneum (*P*), on which scattered cells with tufts of cilia maintain a current towards the free tip of the organ. Within the peritoneal membrane is a slight layer of connective tissue matrix in which lie strong circular and longitudinal muscle fibers

(*Cm Lm*). The connective tissue also forms numerous longitudinal ridges projecting far into the lumen of the tube and containing a few longitudinal muscle fibers (*Lm*). The entire inner surface is covered by a single layer of large irregular cells resembling the chloragogue cells, and often full of clear, homogeneous yellow spherules 4 to .4μ in diameter, which give the characteristic color to the nephridium.

At the nephrostome these lining cells are replaced by small cuboidal cells (Fig. 5), each with a tuft of cilia and gradually passing into the peritoneal cells. The passage from the posterior tube to the exterior through the body wall is also lined by short columnar cells, internally passing gradually into the above granular cells, and externally becoming continuous with the epidermal cells of the body. The cuticle follows these latter cells in, but disappears before they give place to the large granular cells.

In no case were eggs, spermatozoa, or other solid bodies found in the nephridia, though the action of cilia on the flap of the nephrostome appears to collect the eggs at that point. Possibly the contraction of the organ forced out its contents before the examination!

The number and distribution of the colored spherules in the cells varies much, probably in connection with phases of activity of the cells, and in some cases clear vesicles are found, in sections, protruding from the outer parts of the cells, breaking off and occupying the lumen of the nephridium. This suggests what has been observed by Shipley (8) in Phymosoma, which is, however, in my sections due, I judge, to the action of reagents, since the cells are very delicate and on teasing readily break up into spherical masses.

In animals injected with carmine as well as in those fed upon carmine no indication of carmine granules was found in the nephridia.

Retractor Muscles.

These four long muscle bands are somewhat flattened from above downward, and hence have at their origins from the body wall an extension from side to side greater than from before back (Fig. 1). At their insertion they unite to form a continuous muscular sheath in the wall of the pharynx, which is then con-

tinued as the thin, longitudinal, internal muscular layer of the body wall in the introvert. Though entirely free in the body cavity for the major part of its extent, each retractor is anteriorly loosely bound to the œsophagus by innumerable radiating connective tissue strands along one line facing the œsophagus.

The retractors are covered by the usual peritoneum with scattered ciliated cells, and composed of closely packed longitudinal fibers agreeing with the largest fibers of the longitudinal muscles of the body wall.

The fibers are separated by a small amount of connective substance, forming a sheath for each, and containing nuclei. Through this run numerous nerves.

Though normally the two ventral or the two dorsal origins are equidistant from the mouth, yet cases are not rare (as already observed by Keferstein (5 and 6)) in which one of the ventral muscles arises anterior to the other.

Being continuous with the longitudinal muscles of the body wall, both anteriorly and posteriorly (Fig. 2, *VR LM*), and agreeing in structure with them, the retractors may be considered as isolated parts of the body wall musculature, connected with more perfect introversion of the anterior region.

The Reproductive Organs.

As has already been stated in a preliminary note (9), the eggs and spermatozoa found free in the body cavity may be traced to a single ovary or testis which forms a transverse ridge on the posterior face of each ventral retractor (Fig. 1, *R O*) near its origin and extends across the median line ventral to the nerve cord. This ridge is a mass of reproductive cells supported by a connective tissue lamella, which sends branches into the chief digitate projections that give the organ an irregular funibriated surface (Fig. 2, *Ov*). These processes contain the largest cells at the tip, where they break out into the body cavity (Fig. 28), while towards the base and area of attachment of the organ to the retractor the cells are smaller and have no definite walls, though their nuclei are prominent and quite different in size and staining properties from the nuclei of the peritoneum (*P N*), covering the entire organ except where ruptured.

The organ is thus an outgrowth of the connective tissue of the

retractor covered over by reproductive cells that may be regarded as arising from the thickened peritoneum, though perhaps they have no true connection with it, but are in origin, as in position, retroperitoneal.

The ovary is distinguished by the size of the largest terminal cells of some of the digitate processes (Fig. 28), these being as much as 24μ, as opposed to the largest cells observed in the males, measuring 14μ.

Most of the individuals examined from July 20 to September 20, and in April of the following year, presented the reproductive organ in the same condition, as well as eggs and spermatozoa free in the body cavity, some of which cells were apparently mature.

The smallest specimen found, 13 mm. long, contained no sexual cells nor indication of reproductive organ.

Nervous System.

The ring nerves and plexuses of the body wall and the plexus of the digestive tract having been described, there remain the ventral nerve cord, the circumpharyngeal commissures, and the brain. The ventral nerve cord (Fig. 1, *In*) lies free in the body cavity and surrounded by peritoneum, but attached to the body walls by the lateral nerves. In the introvert the cord is flat and wide, and not as near to the body wall as in the posterior region, where much less flattened dorso-ventrally. Anteriorly the cord ends ventral to the pharynx by bifurcating to form the two large circumœsophageal commissures passing to the brain, while at the extreme posterior tip of the animal the cord, diminishing in size, ends in a single slender nerve, or often in two more or less unsymmetrical right and left nerves, passing posteriorly into the body wall, and representing one or two lateral nerves directed posteriorly instead of outward. The other lateral nerves arise in great numbers along the entire cord right and left (Fig. 22), but with no indication of a metameric arrangement or of regularity of any kind: passing first outward they soon bend ventrally and enter the body wall to become the starting-points of the large ring nerves (Fig. 33) between the two muscle layers of the body wall. The ring nerves are thus continuous from one side of the cord to the other around over the dorsal aspect of the body, but would be interrupted on the area ventral to the nerve cord except for

the existence of numerous connecting nerves (Fig. 22, *Cn*) completing the rings by passing from a lateral nerve of one side to a lateral nerve of the opposite side between the two muscular layers. These ventral nervous connections are not as numerous as the dorsal parts of the ring nerves, many of which are thus incomplete ventrally. The size as well as arrangement of the lateral nerves is very variable, while anastomoses between adjacent ones are common in the body wall where the nerve rings start (Fig. 22).

The peritoneum forms a loose sac around the cord and its lateral branches, within which a large mass or sheath of loose connective tissue matrix with free cells and fibers fills up the intervening space between peritoneum and nerve substance. In the matrix several nerve fibers run out in each lateral nerve trunk (Fig. 22, *L N*), anastomosing with one another freely, and give rise from a fusion in the body wall to several ring nerves. Thus each lateral nerve gives rise to several ring nerves.

The nerve cord (Fig. 27) is provided with a well-developed longitudinal muscle (*D M*) running in the connective tissue substance, dorsal to the nerve substance, as a single median mass throughout the posterior part of the body, but dividing at the commencement of the introvert into a right and a left muscle, soon divaricating and passing to positions right and left of the nerve substance. Hence in the introvert there are two longitudinal muscles, one on each side in the transversely expanded nerve trunk. In this region the antagonistic action of these two muscles appears to account for the straight course of the nerve trunk (excepting the vertical bends that occur); while the single posterior muscle throws the nerve cord into transverse bends that are frequently very numerous and abrupt. Abrupt bending of the nerve trunk seems here to have no such evil effect as is required on the supposition that annelid giant fibers are necessary supports in vermicular motion.

The posterior termination of the muscle is by a separation of its fibers into two sets passing posteriorly to the body wall along the two terminal nerves generally present; the anterior termination of the two nerve muscles is by entrance with the two commissures into the ventral retractors, where these are continuous with the body wall. At this region the two lateral

nerve muscles pass up from the sides to the dorsal aspect of the nerve trunk again and then fuse into a single mass just posterior to the division of the nerve trunk into the two commissures lying in the muscular mass about the pharynx.

The structure of the nerve cord as seen in transverse section (Fig. 27) is as follows: Externally the thin peritoneal membrane, then a thick sheath of connective tissue (*C*) with fibers and nuclei in a transparent matrix, and one or two bundles of muscle fibers (*DM*), dorsal or lateral to the central nerve mass, as the case may be. The central nerve mass is enclosed in a special membranous part of the connective tissue sheath, and is traversed in all directions by a spongy meshwork continued inward from this sheath, especially from the dorsal and ventral aspects. The nerve cells are aggregated in the ventral part of the nerve mass in such a way as to suggest a simple epithelium, many of the cells being columnar, resting by the base upon the membrane ventrally, and sending off long processes dorsally into the nerve mass. Amongst these cells there are groups of a few much larger ones (*Gc*) right and left of the median line, which do not form, as do the smaller cells, a continuous ventral sheet, but are separated by considerable intervals, so as to suggest special ganglionic centers and a metameric division of the cord. These cells, however, vary much in size and appear to correspond, in small groups, to the origins of the lateral nerves, and thus to be rather irregularly arranged and not definitely metameric. Some of these larger cells are multipolar as seen in maceration, but have one chief process extending dorsally into the nerve mass. The interstices of the connective tissue framework are occupied by nervous substance, appearing on longitudinal section to have a tubular structure, each clear tube surrounded by connective tissue and composed of a substance resolvable into delicate longitudinal fibrils.

The lateral nerves arise high up on the sides of the nerve mass and have a continuation of the special connective tissue investing membrane with many nuclei, while enclosed (in groups) in lateral outgrowths of the thick connective tissue sheath. The nerve itself contains some connective tissue ingrowths, is finely granular on section, and has in it a few nuclei, apparently connective tissue cells, at intervals.

The Brain.—This is about 2 mm. in diameter and has a somewhat heart-shaped outline (Fig. 6) viewed from the dorsal side, being convex posteriorly and swelling out anteriorly into two prominent lobes. The dorsal and ventral surfaces are flattened and appear roughened by the presence of numerous papilla-like outgrowths (*Pn*), which on section are seen to be nerve fiber bundles passing out through the peculiar connective tissue sheath (Fig. 30, *S*) present upon these two aspects of the brain. From the convex posterior end come off numerous nerves to the dorsal retractors (Fig. 31, *M N*), while from the anterior convex lobes (Fig. 6, *Cn*) nerves pass out into the cephalic lobe (*Wimperkissen*). The large circumpharyngeal commissures (*Com*) proceed from the lateral aspects of the brain and, surrounding the pharynx, immediately converge to form the ventral cord. Each gives off three chief nerves or collection of nerves: the first at the point of origin from the brain (*A Brn*) supplies the branchiæ dorsally; the second from the posterior aspect of the commissure (*Ph n*) is much smaller and soon breaks up in a meshwork in the walls of the pharynx; the third (*L Brn*) arises about the middle of the length of the commissure and supplies the lateral and ventral branchiæ, and probably also the ventral retractors and pharyngeal walls. Other smaller branches are also given off from the commissures.

The position of the brain may be understood when bearing in mind the relations of the surrounding parts. The four retractors appear to fuse into a single mass about the pharynx and then to become continuous with the inner part of the body wall, where it unites with the end of the pharynx. At this point, however, we find the two lateral retractors fused into a single mass, right or left, but on the dorsal and ventral median lines the muscle mass itself is not continuous, but merely united by connective tissue. In this median interval on the ventral aspect of the pharynx nothing but connective tissue separates the muscle mass of the right from that of the left, but on the dorsal side the brain occupies nearly all the space between the right and left muscle masses (Fig. 30, *R M*), which are connected here only by a few fibers dorsal to the brain (*S*). The brain is then in the connective tissue wall of the pharynx between the right and the left retractor muscle masses. Its ventral surface is directly in contact with

the dorsal blood sac (*Bl s*), in fact it appears to protrude ventrally into the vascular enlargement of this vessel (Fig. 31, *Bl s*), which expands ventral and anterior to the brain before sending off the ring-like sinus anterior to the commissures and the right and left dorsal branches to the dorsal branchiæ. Its dorsal surface is for the most part separated only by the peritoneum and the connective tissue (*S*) from the body cavity, but anteriorly it becomes continuous with the body wall where this ends and the pharynx begins (Fig. 31, *Ep*). At this point, in fact, the brain is continuous with the epidermis of the body wall, though elsewhere sunk ventrally into the wall of the pharynx.

Structure of the Brain.—Some of the fibers of the commissures (Fig. 30, *Com*) extend across the ventral part of the brain and some become incorporated with the rest of the purely fibrous central mass, which is covered dorsally by an investment of ganglion cells (*D G*), while ventrally, as the above cross fibers, it comes close to the blood sinus. This large central fibrous part of the brain extends dorsally into the ganglionic portion as two longitudinal ridges, showing on cross-section as rounded horns of the fibrous ventral area. The ganglionic dorsal portion is thickly supplied with small cells, presumably, in part at least, nerve cells, though probably in part connective tissue. These also extend into the two anterior lobes of the brain, and down along the commissures, and, moreover, cover the posterior aspect of the central fibrous mass (which is thus uncovered by ganglion cells only on its ventral side) (Fig. 31).

The extreme posterior convex lobe of the brain contains numerous exceedingly large ganglion cells (*P G*), which occupy its entire area on transverse section.

The dorsal and ventral sides of the brain are provided with peculiar connective tissue investments (Fig. 30, *S*), intervening between the peritoneum and the ganglionic region dorsally, and between the lining of the blood sinus and the transverse fibers ventrally. This connective tissue is clear and gelatinous when fresh, but contains fibers, and also a few muscle fibers in the dorsal region. Radiating bands of fibers from the central parts of the brain break through this connective tissue investment in numerous places, and form either supporting connections with the above membranes, or else (on the dorsal face) the peculiar

origins of numerous small nerves passing posteriorly to the retractors and anteriorly to the cephalic lobe. These radiating masses form the peculiar papillæ seen in the macerated brain (Fig. 6). In the lateral part of the brain, dorsal to the origin of the commissures, there is on each side a peculiar tubular organ (Fig. 30), which will be described in connection with the cephalic lobe.

Cephalic Lobe.—In the expanded state the body wall posterior to the branchiæ is elevated on the dorsal line into a noticeable ciliated cushion (*Wimperkissen*) which lies dorsal to and in part posterior to the brain, with which it is in connection at its anterior end. This ciliated elevation presents a deep median and two lateral grooves subdividing the entire elevation into four longitudinal closely applied ridges (Fig. 32, *Cl*), sharply defined anteriorly and laterally, but gradually passing into the general level of the epidermis posteriorly as the body wall becomes thinner. At the abrupt anterior end the ridges overhang a horizontal groove, or in some stages of contraction a transverse pit, which may be nearly obliterated in full expansion, except at its extreme lateral ends (Fig. 32, *L P*). These latter parts of this horizontal groove continue posteriorly under the ridges, laterally, as flattened tubes 160μ long, one on the right and one on the left of the median plane (*L P*). From each flat diverticulum of the groove, at the point marked by a cross, a cylindrical tube of epidermal cells passes ventrally and posteriorly into the ganglionic part of the brain for about 100μ. Each tube is bent several times, is about 16μ wide, and presents enlargements near its blind end, the largest 24μ. The epidermal cells of its walls give place to what appear as ganglion cells of the brain, while the ciliated lumen of the flattened diverticula is continued as a mass of coagulated, staining substance filling the tube (Fig. 30). The terminal part contains also a central, highly refracting, clear body, especially evident in cross-sections of the enlargements of the tube, though continuing through part of the twisted portion. No pigment was seen associated with this tubular organ, which otherwise suggests, on cross-section, a visual organ.

The ciliated ridges (Fig. 32, *Cl*) are covered by an elongated epithelium intimately connected with large nerves (*Cn*) coming directly from the anterior dorsal part of the brain and running posteriorly in the ridges (Fig. 31, *Cn*).

Comparisons and Conclusions.

Of the various previous accounts of the anatomy of the Sipunculids, I may here refer only to that of Andreae (10) upon *Sipunculus nudus*, and to the recent paper by Shipley upon *Phymosoma varians* (11), as other papers are either summarized in the above or else rendered of comparatively little value by these more detailed and complete accounts. As might have been expected, the anatomy of *S. Gouldii* agrees closely with that of *S. nudus*, and even with Phymosoma to an extent indicating the fundamental identity of the two forms in all but secondary characters.

Differences between the accounts of *S. nudus* and *S. Gouldii* are partly due, I judge, to differences in method, and especially to the free use of fresh material in the latter case.

In the body wall of *S. Gouldii* there is a less specialized condition of the epidermis than in the other Sipunculid mentioned above, though the glandular bodies are essentially identical with those of Phymosoma. In *S. nudus*, however, a peculiar arrangement of the gland cells is indicated by Andreae, which, however, as seen in specimens from North Carolina, seems to me due to poor preservation, while the real condition is much as in *S. Gouldii*.

The complex peripheral nervous system described in *S. Gouldii* will doubtless be found in other Sipunculids on applying gold chloride staining, as the circular nerves and parts of the subepidermal plexus are already known.

That the tentacle-like processes about the mouth are to be regarded as branchiæ in function and probably in morphology also, seems patent after observation of the rapid circulation of corpuscles that there takes place, especially when bearing in mind that the coloring matter of the corpuscles is probably a respiratory one. Though, as has been suggested, the dorsal blood sac may serve partly to form a reservoir for blood in introversion, yet it must serve as a conveyor of respiratory gases to the body cavity liquid and furnish an important adjunct to the thick body wall in this mediation between the sea-water and body cavity liquid. That the branchiæ also serve as means of bringing currents of water (and food) into the mouth seems probable from the position of the cilia on their concave oral

surfaces and the arrangement along radiating oral grooves. This function would of course be null if, as some have stated both in Phoronis and in Sipunculids, the cilia are on the outside surface, away from the oral; but this seems to me an error arising from study of invaginated specimens, where the positions of the branchial surfaces are apparently inverted!

Examination of fresh and hardened material has shown the presence of a nerve plexus in the walls of the digestive tract, the occurrence of "pseudostomata," and the division of the digestive tract into more numerous regions than have hitherto been recognized. That the nerve plexus is continuous with that of the body wall at the anus has been determined, but its connection near the mouth remains doubtful, though it seems to connect with nerves from the brain.

If the "stomach" should prove to be a sucking stomach, as its structure suggests, we would be able to see how such large quantities of sand may be swallowed; though the mere act of crawling would, probably, as in Balanoglossus, tend to produce a forced ingestion.

The structure of the ciliated groove of the intestine suggests a secretory function and at the same time a use as a conduit from one region to another. As the various animals in which such ciliated grooves and a "nebendarm" are found seem to be those addicted, like Sipunculus, to swallowing quantities of sand or other foreign bodies along with the food, the primitive value of such organs may have been associated with the hindrance so caused in the ordinary exchange of the intestinal contents from one part to another. May not then the groove, and subsequently the "nebendarm," have been chiefly secretory and conductive, rather than respiratory, as suggested by Eisig?

There seems no longer any doubt that the reproductive organs, as described in *S. Gouldii*, are typical for the Sipunculids in general, though that this has not been long recognized appears from the following review of work upon this subject.

In 1837 Grube (12) observed eggs in the body cavity of *S. nudus*, from May to August, at Naples, in the *nephridia* also, in November; while at Palermo eggs were observed in the body cavity in June, but not in January. Similarly Delle Chiaje had previously observed eggs in the body cavity during the winter.

In 1849 Meyer (13), observing what seems to have been a clot in the body cavity full of ova, referred the formation of these latter to the body cavity rather than to the nephridia, as had been done by others.

The true meaning of the nephridia in reference to the sexual products was seen by Peters (14) in 1850, when he interpreted them as oviducts, not as ovaries, though his localization of the true ovary as masses projecting into the intestine, but discharging by a slit into the body cavity, seems to have been due to an entirely erroneous observation upon the intestinal groove.

In 1851 Pourtalès (1) discovered *S. Gouldii* and observed the body cavity to be full of what he considered to be eggs, some of which were present in the nephridia also. In 1847 he had seen spermatozoa in the body cavity, and regarded them as formed there. The ovary, however, he sought to identify as what is now known as the blood sac.

In the former year Krohn (15) for the first time observed ripe spermatozoa in the body cavity of *S. nudus*, at Naples, and regarded the sexes as separate in this species and in Phascolosoma. An attempt was made by him to artificially fertilize the ova in April, but this was unsuccessful, owing, as he supposed, to the small number of ripe spermatozoa.

In 1862 Keferstein (16) noticed much disparity in the relative abundance of males and females of Phascolosoma; only two individuals with spermatozoa among 200, most all full of eggs. He states that the smallest species have the largest and at the same time the least numerous eggs.

Claparède (17), in the following year, found also only one male with 112 females, and this one of a different species. He was able to recognize the males by the milky white color due to spermatozoa in the body cavity. The spermatozoa were traced to "floating testes" or cell masses in the body cavity, and a like origin was inferred for the ova. The nephridia were now assigned an excretory function.

Two years later Semper (18) searched in vain for the reproductive organs in Sipunculids, and concluded they were very small and early set free their products into the body cavity. He demonstrated the internal opening of the nephridium, and showed the so-called ovary of Peters to be an intestinal groove.

In 1865 and 1867 Keferstein (5, 6) described the eggs of *S. Gouldii* found in the body cavity, and retracts his previous belief in the hermaphrodite character of the Sipunculids derived from observations of spermatozoa in the nephridia and ova in body cavity.

In the latter year Jourdain (19) observed the sexual products floating in the body cavity in Sipunculus, but was unable to find any organ producing them, though certain pedunculated vesicles on the terminal part of the intestine might be interpreted as such. He also saw the internal opening of the nephridium in three species, but was not able to abandon the old idea of a terminal pore at the posterior end of the body in the case of *S. gigas*.

Brandt (20) in 1870 finds the sexes of *S. nudus* and Phascolosoma about equal in number, with swimming testes and ovaries. The spermatozoa arise by division of primitive spherical cells in the body cavity, and the ova have a similar origin.

A new origin for the reproductive products was discovered by Lankester (21) in 1873 for *S. nudus*. Arborescent tufts growing out from the peritoneum enclose ova, which break loose, singly or in groups, and develop farther, floating in the body cavity. These tufts, however, are situated on each side the rectum, yet when attached he "never found the villi containing unmistakable ova." He was uncertain whether the testis was the nephridium or diverticula of the intestine, but thinks that the testes are still to be sought, since he believes the floating testes of Brandt are really stages in formation of red blood corpuscles. The nephridia were found much distended by pervisceral liquid in May, in which were ova and also spermatozoa-like bodies!

In 1874 artificial fertilization was again tried by Selenka (22) in various species of Phascolosoma, but only two successful results were obtained, and these in a species in which the body cavity liquid does not clot.

Ludwig (23) states that Semper, in his lectures previous to this date, had taught that the genital gland in the Sipunculid was an irregular mass at the base of the retractors. This organ was first well described and figured in 1875 by Theel (24), for *Phascolion Strombi*, as a fringed ridge passing from dorsal to ventral retractor and along the side of the nerve cord. It is alike in both sexes, and lets loose its products into the body cavity: the spermatozoa

seem formed in place as their tails project from the ridge. The males are recognizable by lighter color and smaller size, and occur one to three or four females. The sexual organ was found in September, while the body cavity contained eggs in June, September and February.

In the same year (25) he describes the reproductive organs of *Ph. boreale* as paired and posterior to attachment of ventral retractors.

In 1877 Spengel, as quoted by Andreae, found the reproductive organs in Sipunculus as a transverse band of small cells at the base of the ventral retractors, while Koren and Danielssen (26) found no reproductive organs or products in two species of Sipunculus examined in midsummer. They did, however, find both in many species of Phascolosoma. Spermatozoa, however, were found only in two individuals. As to the reproductive organ itself, the authors have seen and figured it in *Ph. Loveni*, *Ph. abyssorum*, *Ph. eremita*, *Ph. margaritaceum*, *Ph. Harveii*, and *Ph. papillosum*. Though correctly figured, unless it be that in many cases the organ passes ventral to, in others dorsal to, the nerve cord, contrary to what one would expect to be the case, these organs are not considered reproductive organs at all, but of unknown function. What they do believe to be reproductive organs are described as masses attached to the œsophagus, apparently, in fact, the blood sac again, though in some of his figures the so-called generative organ strongly suggests a clot of coelomic liquid. In supposing that the conical posterior part of the body bursts to allow the ova to escape, the authors again revert to an older notion regarding the reproductive processes.

Two years later Cosmovici (27) finds the sexual organ in both sexes of *Ph. vulgare* as botryoidal masses at the base of the posterior retractors.

Sluiter (28) in 1881 found reproductive organs in various tropical Sipunculids: in *S. edulis* and in *Ph. nigritorquatum* the ovaries are a series of sausage-shaped sacs between the dorsal retractors, out of which the eggs escape by rupture of the walls; in *Aspidosiphon fusca* and in *Ph. falcidentatum* the organs are open troughs, from the walls of which the ova escape immediately into the body cavity. The testis is recognizable as smaller and lighter in color, but was seen only in *A. fusca*. These organs

are not to be confounded with those assumed by Teuscher in regarding the sexual products as arising in the channels of the body wall. The variation in the character of the reproductive organ is extended also to the nephridium, which was found in some species opening internally near its posterior, free end; in others anteriorly, as described in *S. Gouldii*, etc.

Selenka (7) in his monograph upon the Sipunculids in 1883–1884 includes the possession of sexual organs as ridges at the bases of the ventral retractors among the characters of the group, and states that he has seen such organs in several species of Phascolosoma, where they form a ruffled band with small ova seen in sections and upon teasing.

Finally Shipley (8, 11), within the past year, has shown the reproductive organs of *Phymosoma varians* to be identical with those of *S. Gouldii;* and in general this is the conclusion to be drawn from the more detailed and recent of the above accounts. Moreover, sections of poorly hardened *S. nudus* have shown me that here also the reproductive organs have the same structure as in *S. Gouldii.*

In the central nervous system of *S. nudus* the close agreement of the brain in its structure with that of Phymosoma is interesting as suggesting some direct connection between the peculiar locomotion and introversion and the presence of giant ganglion cells in the posterior part of the brain.

The peculiar brain tubes also are apparently identical in both. Whether they are to be regarded as eyes, glands, or possibly equilibration organs, I have not been able to determine from *S. Gouldii;* here the structure seems less markedly eyelike than in Phymosoma.

In conclusion I may say that the examination of the adult anatomy of this Sipunculid leads to a better appreciation of the similarities between this group and Phoronis, so that I would concur with Shipley in referring Phoronis to a place close to the Sipunculids, especially as the prevalent connective tissue matrix of *S. Gouldii* might readily be homologized with more special forms of it differentiated as supporting skeleton in Phymosoma and Phoronis.

Moreover, in spite of the lack of discovered metameric structure in the Sipunculids, their close resemblance to the Priapulidæ,

and then to the Echiuridæ, furnishes a ready means of deriving them all from metameric polychætæ-like forms. The changes that have taken place among some sedentary annelids with partial loss of metamerism suggest a parallel, as has been emphasized by Wilson and others.

I see nothing in the adult Sipunculid that may not be explained on this assumption of lost metamerism; even the reproductive organs suggest a derivation from those of polychætæ, with which they agree in structure and fate very closely. Their attachment to the posterior side of a septum may now be indicated by their attachment to the retractors. On the other hand, the direct derivation from non-metameric larval or other ancestral forms would necessitate much complex parallel variations in Sipunculids and Echiurids.

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EXPLANATION OF PLATES.

All the figures are from camera drawings with Zeiss lenses, unless otherwise stated.

PLATE XLIV.

FIG. 1. Body wall cut along right side and spread out to show general arrangement of organs. About natural size; free-hand drawing. *Br*, branchiæ; *In*, nerve cord, flattened in the introvert; *R D R*, right dorsal retractor; *R N*, right nephridium; *Oe*, œsophagus; *R V R*, right ventral retractor; *R O*, right end of reproductive organ; *I M*, mesentery of ascending intestine; *R*, rectum, with lateral ending of mesentery; *R C*, rectal diverticulum or cæcum; *Bl S*, end of blood sac.

FIG. 2. Vertical longitudinal section of body wall and end of ventral retractor (*V R*), to show reproductive organ (*Ov*) in section. *L M*, longitudinal muscle; *C M*, circular muscle of body wall; *Ct*, cuticle over the epidermis, with glands projecting from the latter into the sub-epidermal tissue. 2 A ½.

FIG. 3. Transverse section of the body wall. *Ct*, cuticle; *Ep Gl*, orifice of granular epidermal gland; *Ep Gl'*, same of the many-celled gland; *Ep*, epidermal cells; *Sep*, sub-epidermal connective tissue, with part of nerve plexus; *C M*, circular muscle; *L M*, longitudinal muscle, part of the nerve plexus shown between the two muscles; *P*, peritoneum; *B C*, body cavity, with grooves extending into longitudinal muscle. 2 D ½.

FIG. 4. Transverse section of the mesentery of the ascending intestine. *n*, central nerve of muscle; *Mf*, muscle fibers; *Con*, connective tissue matrix with scattered cells, and fibers in the peripheral end around the muscle; *Chc*, chloragogue cells over outer surface of mesentery; *I W*, wall of intestine.

FIG. 5. Longitudinal vertical section of the body wall and nephridium, to show the external opening (*N M*) and the internal nephrostome (*Np*). *In*, internal chamber of nephridium lying *anterior* to the large terminal chamber (*Ex*), with which it communicates around end of the intervening partition. Other letters as above. 2 A ½.

PLATE XLV.

FIG. 6. Dorsal view of brain and nerves, from macerations. *A Brn*, three anterior nerve trunks to branchiæ; *L Brn*, two lateral nerve trunks to branchiæ; *Ln*, lateral nerve of ventral cord; *Com*, circumpharyngeal commissure; *Cn*, nerves from brain to the cephalic lobes; *Pn*, posterior nerves from brain to pharynx; *Phn*, pharyngeal nerve from origin of the commissure. 2 A ½.

FIG. 7. Cells of epidermal organs. *A*, two cells from the many-celled gland, showing a vacuole-like secretion (*V*) and a terminal process (*Pr*) at the inner end; *B*, cells of the sense organs—*a*, central, and *b*, peripheral; *C*, one of the large-grained cells of the few-celled epidermal glands. 2 F ½.

FIG. 8. Silver nitrate preparation of part of peritoneum over intestine, showing outlines of cells and one of the pseudostomata cells, *PSC*. 2 F ½.

FIG. 9. Part of the nerve plexus on the inner surface of the longitudinal muscles next the peritoneum. Gold chloride preparation. 2 D ½.

FIG. 10. Optical section of some of the radiating fibers of the walls of the stomach, showing the central nucleus. 4 D ½.

FIG. 11. Surface view of the expanded branchiæ and oral disk. *Cl*, cephalic lobe on median dorsal line. × 25 diameters.

FIG. 12. Diagram of the arrangement of the branchiæ on an infolded membrane forming symmetrical oral ridges. The numerals indicate the number of branchiæ upon successive infoldings. *D* and *V*, dorsal and ventral median ridges; *DL* and *VL*, dorso- and ventro-lateral chief ridges.

FIG. 13. Transverse section of the stomach to show the four great ridges of radiating fibers reducing the lumen to an X-shaped series of grooves. *Rf*, radial fibers; *Mf*, one of the mesenterial fibers connecting stomach with intestine; *P*, peritoneum covered by chloragogue cells. 4 A ½.

FIG. 14. Spermatozoa (*S*) and portion of surface of ovum (*O*) seen in section. *Yk*, yolk; *Z*, membrane with radial tubes; *P*, outer coating of ovum with pseudopodia-like processes passing out through tubes of egg membrane. 4 F ½.

FIG. 15. Corpuscles from body cavity. *Gc*, giant corpuscles seen from the side and from the edge—2 F ½; *C'*, large-grained amoeboid corpuscle; *C''*, small amoeboid corpuscles; *C*, colored corpuscles from side and edge views. 4 F ½.

FIG. 16. Colored corpuscles of the dorsal blood sac. 4 F ½.

FIG. 17. Longitudinal section of one of the intestinal diverticula. *P*, peritoneum; *Rf*, radial fibers.

FIG. 18. Longitudinal section of the spindle muscle connected by the connective tissue part of the mesentery to the spiral ascending intestine, which is cut twice transversely. *CG*, ciliated groove of intestine; *N*, nerve in the spindle muscle; *P*, peritoneum and chloragogue cells over the mesentery; *M*, muscle fibers of spindle

muscle; *PS*, pseudostoma amongst the chloragogue cells over the intestine. 2 A ½.

PLATE XLVI.

FIG. 19. Gold chloride preparation of the nerve plexus in the walls of the digestive tract. The chief trunks are longitudinal. 2 D ½.

FIG. 20. Transverse section of part of wall of nephridium showing two of the ridges projecting into its lumen. *P*, peritoneum; *Cm*, circular muscle fiber; *Lm*, longitudinal muscle fiber. 2 F ½.

FIG. 21. Longitudinal section of part of wall of intestine to show ending of radial fibers (*Rf*) by branching among ends of epithelium cells (*e*). *Chc*, chloragogue cells; *Psc*, transverse section of the funnel of a pseudostoma; *f*, circular fibers cut across; *f'*, longitudinal fibers; *n*, a longitudinal nerve of the intestinal plexus. 4 D ½.

Fig. 22. Ventral view, from maceration, of gold chloride stained nerve cord and lateral nerves. *Ln*, lateral nerve arising as several fibers in a connective tissue sheath and distributed in walls of body as numerous ring nerves; *Cn*, ventral commissure uniting ring nerve of one side with that of other side, and passing ventral to cord in body wall. 2 A ½.

FIG. 23. Section of outer part of wall of intestine to show a pseudostoma and chloragogue cells. *Psc*, crescentric cell with nucleus cut in half, guarding opening into funnel, occupied by a granular cell (*G*); *Chc*, chloragogue cell with large granules; *f*, fibers of wall internal to peritoneum. 4 F ½.

FIG. 24. Transverse section of ciliated groove of the ascending intestine when open. 4 D ½.

FIG. 25. The same when nearly closed. *Rf*, radial fibers. 4 D ½.

FIG. 26. Gold preparation of part of sub-epidermal nerve plexus. The larger trunks are longitudinal. 2 D ½.

PLATE XLVII.

FIG. 27. One half of a transverse section of the ventral nerve cord. *C*, connective tissue sheath around the nerve substance; *DM*, dorsal muscle in the sheath; *Gc*, large ganglion cells in the layer of ganglionic cells on the ventral aspect of the nerve substance. 4 D × ½.

FIG. 28. Section of terminal part of a process of the female reproductive organ, showing the large ova and the flat peritoneal investment. *Pn*, nucleus of peritoneal membrane. 2 F × ½. At the left the ova are ready to break loose.

FIG. 29. Section of part of wall of descending intestine to show a ciliated pit and the granular cells lining the intestine. *N*, part of nerve plexus amongst the radial fibers (*Rf*); *f*, circular fibers near basement membrane. 4 F ×½.

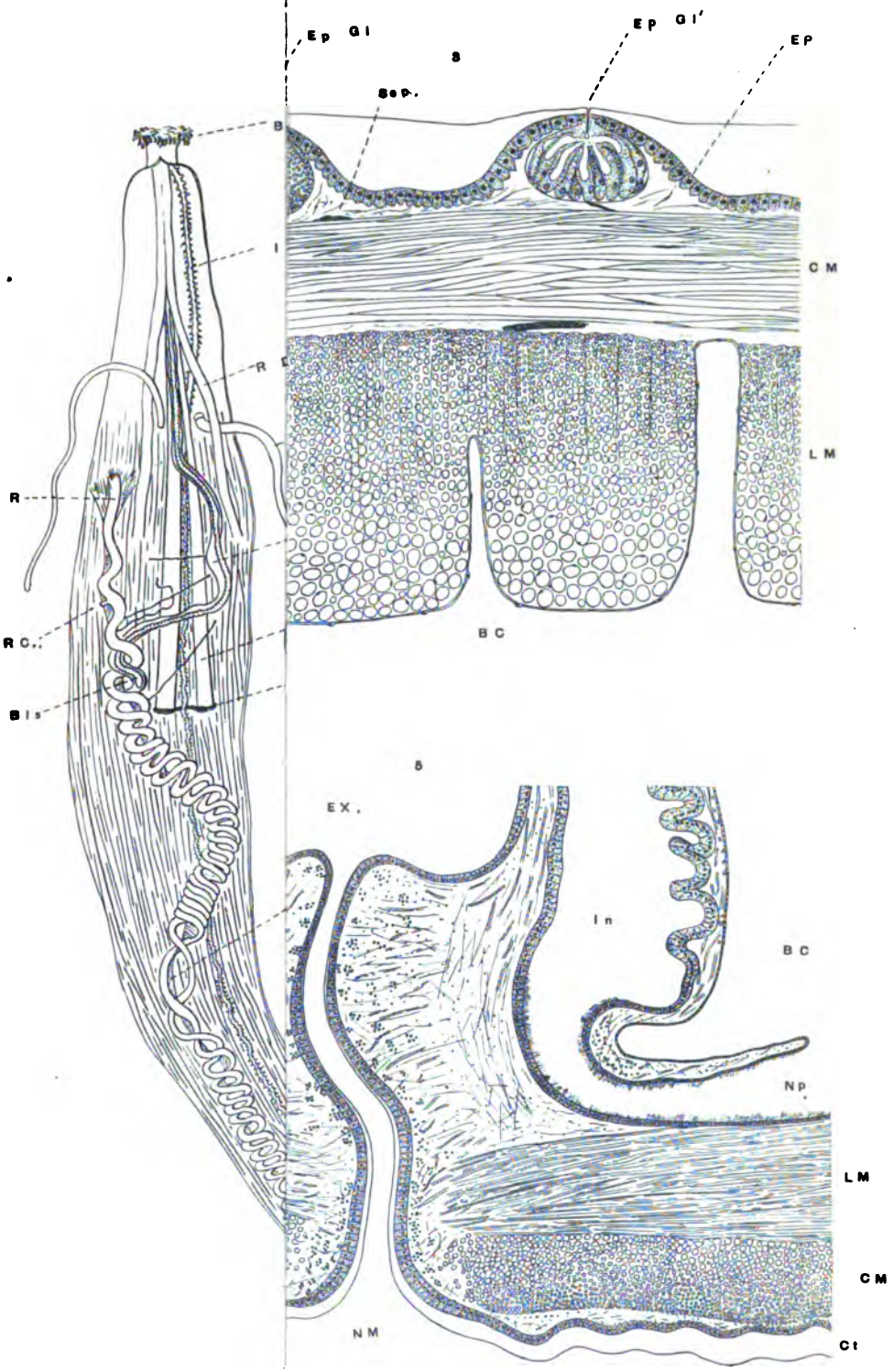
FIG. 30. Transverse section of one half of brain. *BC*, body cavity; *Bls*, blood sinus; *Com*, commissure passing into fibrous ventral part of brain; *DG*, dorsal ganglionic investment of brain containing one of the two dorsal horns of fibrous substance, external to which is shown the brain tube in cross-section, with its central refracting contents; *RM*, retractor muscle mass on side of brain; *S*, connective tissue investment of brain, dorsal and ventral. 2 D ×½.

FIG. 31. Longitudinal section of the brain near and parallel to the median plane. *Cl*, cephalic lobe cut longitudinally; *Cn*, cephalic nerves; *Ep*, epidermis dorsal to brain and directly continuous with it; *DG*, dorsal ganglia cells; *PG*, large posterior ganglia cells; *BC*, anterior termination of body cavity on median plane; *Bls*, blood sinus ventral and anterior to brain; *RM*, retractor muscle mass posterior to brain receiving nerves (*mn*). 4 A ×½.

FIG. 32. Transverse section of one half of brain and cephalic lobe at point of origin of commissures, but anterior to Fig. 30. *Cl*, one half of cephalic lobe showing the lateral groove and branching cephalic nerves (*Cn*); *Lp*, lateral pit extending horizontally ventral to cephalic lobe, connected with its fellow immediately anterior to this section across median line (where the epidermal cells are seen cut tangentially). At the outer corner (†) this pit penetrates the brain in more posterior sections, as the brain tube (Fig. 30). *A Brn*, anterior branchial nerves cut at point of origin. Other letters as in Fig. 30. 2 D ×½.

FIG. 33. Diagram illustrating arrangement of nerves in body wall. *Vc*, ventral nerve cord giving off lateral branches through the longitudinal muscles (*LM*); *LM*, longitudinal muscles pierced by the lateral nerves from cord, which then form ring nerves giving back radiating nerves to a plexus on inner face of longitudinal muscles; *CM*, circular muscles pierced by radiating branches from the ring nerves and covered externally by the sub-epidermal plexus; *Ep*, epidermis of polygonal cells removed, except at right end, in order to show the sub-epidermal plexus.

BALTIMORE, *May* 30, 1890.



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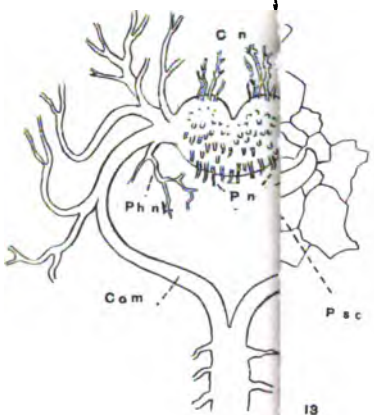

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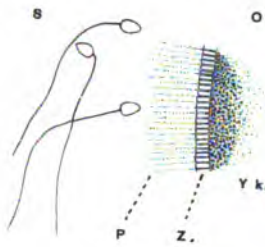

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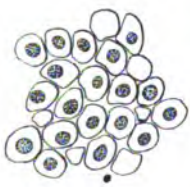

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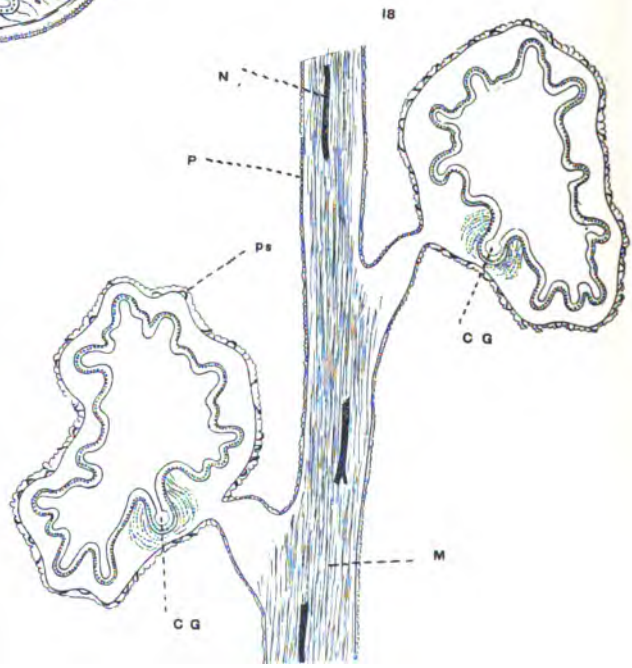

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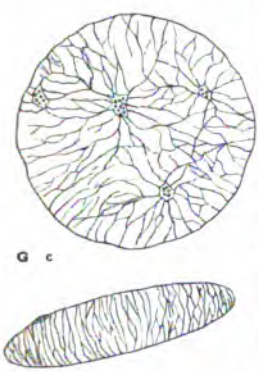

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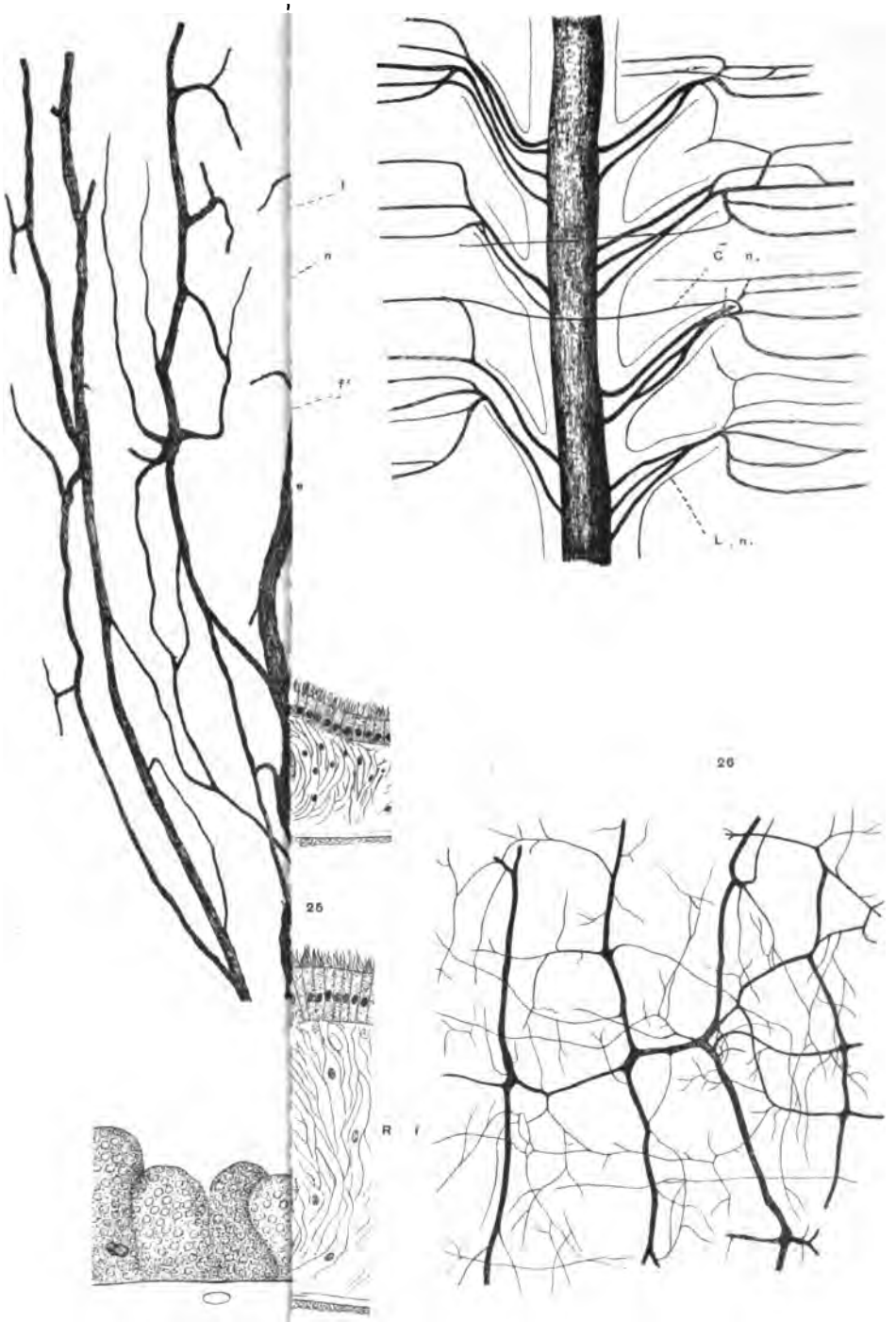

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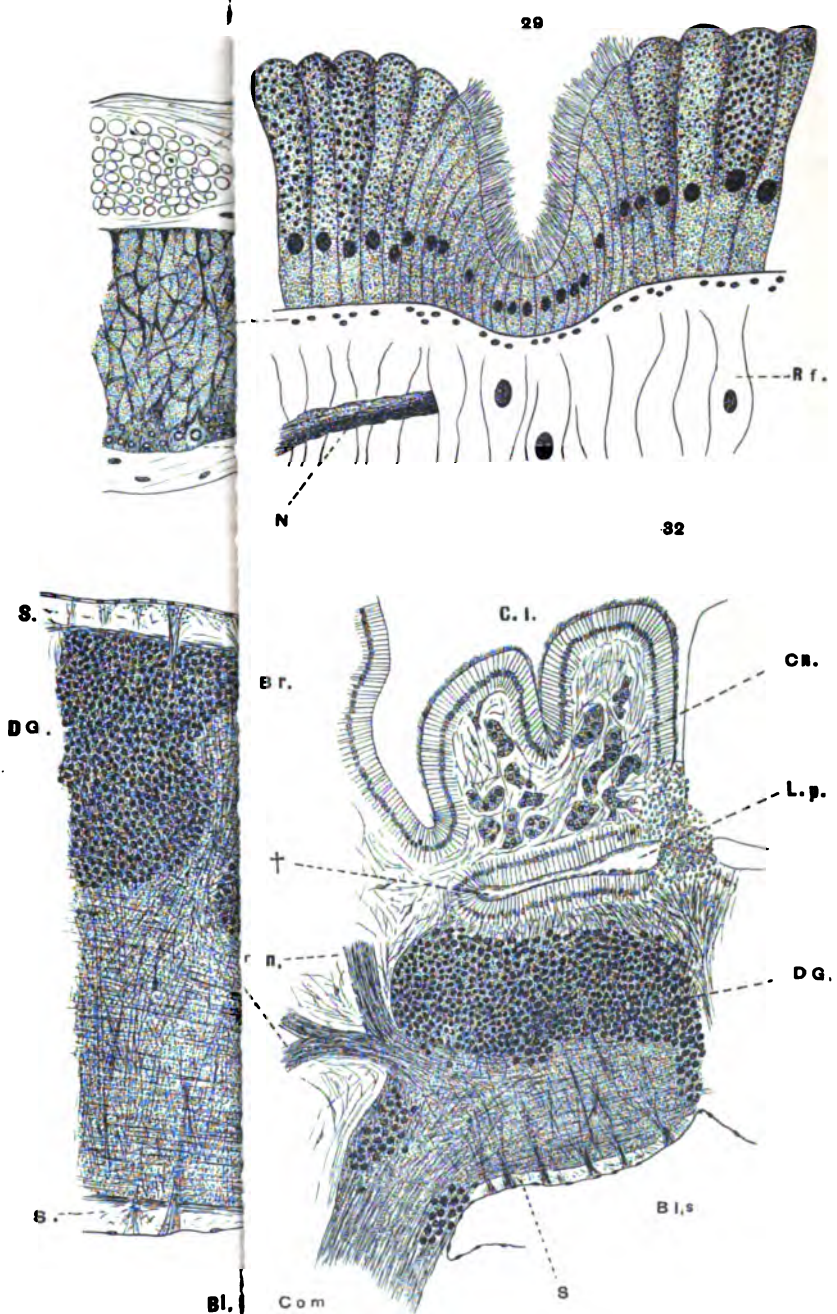

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THE RELATIONSHIPS OF ARTHROPODS.

By H. T. FERNALD. With Plates XLVIII, XLIX, L.

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INTRODUCTION.

The problem of the phylogeny of Arthropods has recently been attracting much attention among biologists. Most observations, however, have been upon the Crustacea, Limulus, Peripatus and the Arachnids, nearly all writers contenting themselves with a few statements of the "probabilities" concerning the Hexapods and Myriapods.

The theory of Brauer and Lubbock, that the insects are derived from a Campodea-like ancestor, was in a great measure the result of the discovery that in widely separated groups Campodea-like larvæ occur, and the theory was formulated almost entirely from external features. Important for the acceptance or rejection of this belief, therefore, as is the study of the anatomy of larvæ and of the Thysanura, to which group Campodea belongs, almost nothing in this line has been attempted, and the scattered

papers forming the literature of the subject abound in inaccuracies and wrong inferences.

The group Thysanura as given by Claus ('85) really consists of two—the Cinura, including the genera Campodea, Japyx, Machilis, Lepisma, etc.—and the Collembola, a larger group including the genera Smynthurus, Tomocerus, Podura, Lipura, etc. To these two has recently been added a third—the Symphyla—containing the single genus Scolopendrella.

The anatomy of most of the Cinura is now quite well known, thanks to the patient and careful labors of Grassi, Oudemans and others, but for the anatomy and histology of the Collembola I have been able to find but one recent paper—an article upon Macrotoma (Tomocerus) by Sommer ('85).

Our ignorance of these groups and the value of a knowledge of them for the student of the phylogeny of Arthropods has induced me to devote my attention chiefly to these forms. My studies have been upon a degenerate Collembolan, Anurida maritima, and upon the Cinuran, Lepisma saccharina. I shall here give the results of these studies, following which I shall endeavor to give a summary and consideration of the most prominent views as to the phylogeny of the Arthropods and especially of the Hexapods, examining some portions by the light of the studies just preceding. Finally I shall sum up the conclusions reached and offer a few suggestions as to the origin of the group and the possible paths along which its main branches may have developed.

Anatomy of Anurida maritima, Guer.

This little animal occurs along the eastern and the western shores of the Atlantic Ocean. It has been reported from Havre (Laboulbéne, '64) and Tréport, France (Nicolet, '47), from St. Andrews, Scotland, and Kinsale, Ireland (Lubbock, '73), and in this country from Nantucket, Mass. (Packard, '73), and I have myself found it abundant at Annisquam, Mass., on the northern side of Cape Ann. It is found between high and low water-marks, in crevices of the rocks, under small stones, or floating helplessly about in the tide pools, where it frequently forms clusters of some little size. It is slow in movement and is easily captured, and, according to Laboulbéne, feeds on small

mollusks, it being only necessary to place one of these with its shell crushed, near the crevices where the Anurida lives, to obtain large numbers of them collected about the food.

Preparation.

The animals were placed either in an aqueous solution of picro-sulphuric acid to which a little ether had been added, or in Perenyi's Fluid, similarly treated. In the former fluid the specimens were left two hours, and in the latter one hour, after which they were slowly raised through different grades of alcohol to that of 90 per cent. The ether was added to dissolve the fatty matter with which these animals are covered, that the killing agents might penetrate more rapidly.

After a few unsuccessful attempts to stain *in toto*, I found that the best results were secured by placing the animals for about twelve hours in absolute alcohol, then in succession three hours in chloroform, one hour in a solution of paraffine in chloroform, three hours in melted soft paraffine, and finally six hours in melted hard paraffine. These lengths of time were varied, but those here given proved about the best. The specimens were now imbedded and sections cut with a Schanze microtome, to one half of one of the sixty divisions of the drum wheel, or about one four-hundredth of a millimeter.

The sections were fixed to the slides with Mayer's Albumen Fixative, after coagulating which, the slides were passed successively through turpentine, absolute alcohol, ninety, eighty and seventy per cent alcohol, four minutes in each, then placed for three minutes in Böhmer's Hæmatoxylin. They were then washed in one half per cent alum solution to which an equal volume of ordinary alcohol had been added, and were then passed through the various grades of alcohol in the reverse order; placed in turpentine for several minutes, and then mounted in Canada Balsam.

External Anatomy.

The adult insect is from 2 to 3 mm. in length, rather linear in form, and of a shining blue-black color, lighter in young specimens.

The body is distinctly divided into head, thorax and abdomen.

The head is rather flattened and pointed in front, so that on side view it resembles a right triangle, the ventral surface representing the altitude, and its line of attachment to the thorax, the base. On the dorsal surface of the head, in front, are the two antennæ, simple and usually three-jointed, though I have observed four joints in a few instances (Fig. 46). Lubbock ('73) states that mutilation of the antennæ is extremely common in this group, and that a reproduction of the lost part is frequent at the next molt. If so, this will explain the different number of joints in different individuals; but as the terminal joint in those having but three, is in form and size like the terminal joints of those having four, besides having well developed sense organs, I am doubtful whether mutilation is the cause of this variation.

The terminal joint is usually somewhat larger than the others, and bears on its tip a cluster of bristles and on its dorsal inner surface a trilobed sense organ. On each side of the head, just behind the base of the antenna, in a slight depression, is a peculiar structure—the post-antennal organ—first described by Laboulbéne ('64). Just behind this is a cluster of five simple eyes ("ocelli") imbedded in a mass of pigment, denser here than elsewhere.

The mouth (Fig. 3) is nearly terminal, only slightly ventral, and is a circular opening, with a cleft extending back a short distance on the ventral median line.

The thorax consists of the prothorax, mesothorax and metathorax, all distinct, and each bearing a pair of five-jointed legs, ending in a simple claw. There are no lobes or ridges on the dorsal surface of the thorax.

The abdomen consists of six segments. On the under surface of the first segment is the abdominal vesicle or ventral tube, which consists of a somewhat antero-posteriorly elongated protuberance, with a median longitudinal cleft. Passing forward from the bottom of the cleft along the ventral mid-line to the posterior end of the ventral cleft of the mouth is a peculiar tube-like structure. The anus is terminal; ventral to it, and opening on a small papilla of the fifth segment, is the single genital opening. There is no external indication of any structure in the adult resembling the "spring" possessed by most of the Collembola, but in the young, Ryder ('86) describes two processes on

the ventral surface of the fourth segment which he considers a rudimentary "spring."

Integument.

The integument consists of a hypodermis which secretes an external chitinous cuticula, and rests upon a basement membrane (Fig. 2). Its thickness varies, being less on the under surface of the animal, and greatest on the head (Fig. 3), but averaging from 0.03 mm. to 0.04 mm.

The cells of which it is composed show no boundaries, and are nearly filled with a dense black pigment which, in the specimens examined, concealed all nuclei from sight, and which immersion of the sections in 45 per cent nitric acid for ten minutes failed to remove. In young specimens the pigment is less dense and does not fill the cells so completely, thus giving the animal a lighter color.

The cuticula also varies in thickness in different parts of the body, but is usually thinner than the underlying hypodermis. It is transparent and colorless, and is quite thickly covered by somewhat irregularly conical protuberances which show a tendency to an arrangement in rows (Fig. 1). When examined in section, some specimens indicate a partial separation into two layers, one internal, the other external, the line of separation passing just below the bases of the protuberances (Fig. 2). Scattered among the protuberances are long bristles, generally in small groups, many, probably all, sensory in function.

The basement membrane on which the hypodermis rests is apparently structureless, and marks the external limit of the body cavity. In many places this membrane is separated from the hypodermis by the fat body, or, as it is called by Sommer ('85), the "Exkretionsorgane," which attaches directly to the hypodermis, and over the surface of which the membrane is reflected.

In some places the integument undergoes special modifications, especially in connection with the sense organs. In every case, however, the superficial modifications are hypodermic, and the cuticula is secreted as a closely fitting outer coat conforming to this modification, the apparent exception of the post-antennal organ to this not being a real one.

Muscular System.

The muscles are well developed and follow definite courses, with little variation in different specimens. They attach themselves to the cuticula on its inner face, at which points the hypodermis is somewhat modified, losing its pigment, and apparently itself becoming somewhat chitinous (Fig. 6). When the attachments are internal they may be either to the tunica propria or to chitinous bodies, themselves held in place by other muscles passing in various directions to the outer surface.

The muscles either cross or pass along the walls of the body cavity in which they lie. They consist of from one fiber to several bundles, but each fiber is surrounded by a sarcolemma, and when several fibers form a bundle a perimysium is also apparently present.

The muscles differ histologically, forming two classes. In one class the fibers seem very granular, have rather poorly defined outlines, and only faint indications of light and dim bands (Fig. 6). In the other class the outlines are very distinct, and the light and dim bands show plainly, and many fibers distinctly show Krause's membrane bisecting the light bands at right angles to the direction of the fiber (Fig. 8).

Digestive System.

The oral opening leads into a buccal cavity of considerable size, which in longitudinal section (Fig. 3) is seen to divide posteriorly into a dorsal and a ventral chamber. The dorsal chamber is the beginning of the pharynx, and is nearly circular in cross section. The ventral chamber is broad and bent dorsally at the sides. A little behind the point of separation from the dorsal chamber it divides into two pouches, one on each side, which continue backward for some distance, and also bend dorsally. Arising from the inner ends of these pouches, and passing forward into the buccal cavity, are two pairs of jaws, mounted on long hollow stalks. One pair arise dorsal and lateral to the other pair; the four together, when seen in cross section, forming a broadly open V, with its lower angle unmarked.

The homologies of these jaws I have been unable to determine with certainty, but consider the dorso-lateral pair as representing the maxillæ, and the others the mandibles, of the higher insects.

The maxillæ (Fig. 9) are divided at their tips into three processes. Of these the middle one bears irregular blunt teeth, while that on one side has a large number which are long and fine, and that on the other side fewer, shorter, saw-like ones. The three processes are apparently movable, and are probably controlled by muscles which pass within the chitinous shell of the maxilla stalks from their tips to their bases.

The mandibles (Fig. 10) end in a single blade-like process, bearing a few irregular teeth. A muscle here also passes from the tip of the stalk to the base, but its function must differ from that of the maxillary muscle, as the mandibular process seems to be firmly fixed to the stalk, and not attached to it by a joint.

The walls of the ventral chamber and of the two pouches are lined by granular cells, with large deeply staining nuclei. They much resemble gland cells. They are covered externally by the chitinous cuticle lining the mouth cavity and fore-gut, and which is here thin and delicate.

On the dorsal median line of the ventral chamber is a chitinous ridge extending forward to where this chamber joins the buccal cavity. The arrangement of the neighboring muscles seems to indicate that this ridge may serve as a sort of fulcrum for obtaining certain motions of the jaws, and as a firm attachment for other muscles running to different parts of the head.

The muscles for moving these jaws are quite numerous, and there are also sets of extensor and retractor muscles to move the chambers containing the jaws themselves forwards, that the latter may protrude from the mouth, and to draw the chambers back into the cavity of the head. In this last position the lateral pouches often extend nearly to the back of the head.

The pharynx bends upward and then backward, extending to the anterior surface of the brain, beneath which it passes through the circum-œsophageal commissure, at the same time changing its structure and becoming the œsophagus. This extends to the posterior end of the prothorax, where it joins the mid-gut, the pharynx and œsophagus together forming the fore-gut.

The œsophagus is lined by a delicate chitinous cuticle which is thrown into irregular folds (Figs. 4, 5). Immediately surrounding it is a granular protoplasmic layer containing numerous round or oval nuclei. Cell walls are either indistinguishable or

nearly so, but longitudinal sections show the cell limits by slight depressions of the cuticle, alternating with the nuclei. The cells rest upon a very delicate tunica propria, which separates them from the layer of circular muscles. Each muscle fiber completely encircles the œsophagus, and the fibers are closely packed side by side and are of the striped variety. On the median dorsal line is the nucleus of each fiber. External to this layer of muscles is a delicate connective tissue membrane, the membrana limitans externa of many writers. There are no longitudinal muscle fibers.

The pharynx differs from the œsophagus only in having its circular muscle layer more developed, and in having dilator muscles—dilatores pharyngis—which attach themselves to the tunica propria, pushing in between the circular fibers and passing to their various points of origin. There are three groups of these muscles, two dorsal and one ventral. One dorsal group passes forward from its attachment, its fibers diverging to the right and left sides and becoming joined to the body wall. The other dorsal group passes directly upward in front of the brain, its fibers also diverging to the right and left. The ventral group runs directly back beneath the pharynx to a point between it and the sub-œsophageal ganglion. Here the fibers attach to a chitinous mass held in place by steadying muscles, which pass in various directions to the body wall. The function of the dorsal groups of muscles is to pull the pharynx forward and upward, and that of the ventral group to pull it back.

At the hind end of the prothorax the œsophagus enters the anterior end of the mid-gut, into which it protrudes slightly, forming a papilla (Fig. 12). As the mid-gut has about three times the diameter of the fore-gut, and this papilla enters it close to its ventral wall, a dorsal and lateral pocket is formed, which has been homologized with the proventriculus of the higher insects, but, as it seems to me, on very slight grounds. The proventriculus in most insects is separated by a small tube from the stomach, which is the organ homologous with the mid-gut here, and can be easily understood as an enlargement of the œsophagus. If the tube posterior to it and connecting it with the stomach were pushed into the latter to form the papilla, even then the proventriculus would not assume the position of this

pocket, and I am more inclined to consider this structure as the incidental product of the union of the stomodæum and mesenteron and functioning as a sort of valve.

Just at the base of the papilla the circular muscles form a close ring, and constitute what may together with the papilla be termed the cardiac valve.

The papilla consists of elongated, club-shaped cells, the larger ends protruding towards the mid-gut. The cell boundaries are very indistinct. The papilla is evidently a part of the fore-gut, as it is covered by a chitinous cuticle, which ceases, however, at the bottom of the pocket described above (Fig. 12).

The mid-gut extends to the end of the fourth abdominal segment, increasing slightly in its diameter towards its hinder end, but contracting again before joining the hind-gut. Its structure is quite uniform throughout. It consists of a layer of cubical, almost columnar, polygonal, epithelial cells, each cell containing near its center a quite large oval nucleus, showing several chromatin granules (Figs. 11, 13, 14, 15). Many of the cells contain vacuoles, generally near their bases, and some have also a large spot of chromatin-like substance in some part of the cell. The free surface of the epithelium when examined with a Zeiss D. Oc. 4 shows a deep-stained narrow band, but under a one-twelfth inch immersion objective this becomes a narrow border outside the cell boundary, and apparently composed of fine hairs. I am inclined to believe that my specimens were not killed quickly enough to preserve these portions as well as could be desired, and that this border is the "Härchensaum" of Frenzel ('86), which he finds in the mid-gut of Crustacea and insects, and which he apparently considers as one kind of cilia, for he says, "Die Wimperzellen könnte man dann zweckmässig in Flimmerzellen und Härchen- (Härchensaum) zellen eintheilen."

The cell walls vary greatly in their distinctness, being well marked in some and hardly to be seen in others. I am inclined to attribute this latter condition also to poor preservation. The epithelium is not thrown into folds, but lies quite smoothly, and rests on a very delicate tunica propria continuous with that of the fore-gut.

The mid-gut has both circular and longitudinal muscles. The

former are much smaller than those of the œsophagus, and are some distance apart (Fig. 11). Otherwise they are quite like those of the fore-gut. The longitudinal muscles are also very small. They are scattered over the outer surface of the mid-gut, external to the circular fibers, and are very unimportant in its anterior portion. In the posterior portion both layers become more extensively developed (Figs. 14, 15). External to all is an enveloping connective tissue membrane.

The appearance of the epithelium of the mid-gut differs greatly at different times, especially during ecdysis, and these changes will be described later.

The hind-gut extends through the fifth and sixth abdominal segments, and opens to the exterior between a large dorsal papilla and two smaller ventral ones, placed almost at the extreme tip of the body. Where the mid-gut and the hind-gut join, the epithelium becomes columnar and forms a thickened band, external to which is a bundle of circular muscle fibers, forming a valve which may be termed the pyloric valve (Fig. 16).

The epithelium of the hind-gut consists of low granular cells thrown into irregular folds and ridges projecting into the lumen. The cells rest upon a tunica propria which is very delicate. External to this are scattered circular muscles, which do not follow the epithelium in its foldings, and thus spaces are left between them and the tunica propria (Fig. 17). The epithelium contains large oval nuclei, showing several chromatin granules. On the surface bordering the lumen is excreted a chitinous cuticle, bearing neither hairs nor spines.

The circular muscle fibers which are attached to the tunica propria at intervals, are of the striped variety, and, as in the fore-gut, each fiber completely surrounds the gut. External to these muscles is a connective tissue membrane, which bends down on each side of the muscle fiber till it touches the tunica propria.

In these hollows blood corpuscles may frequently be observed, and Sommer, who observed a similar structure in Tomocerus, suggested that aeration of the blood takes place here. On the other hand the cavities appear to form a part of, and to be in free communication with, the general body cavity in which blood corpuscles are abundant, and for this reason I see no indication that we have here to deal with any special mechanism more than

anywhere in the gut. It seems to me that this relation of the muscles to the epithelium secures a more complete closure of the lumen, thus aiding in the expulsion of the fæces, and accomplishing this more effectually than if the muscles were attached continuously.

No longitudinal muscles occur, but from various points on this portion of the gut, muscles—dilatores recti—pass dorsally, laterally and ventrally to the body wall, thus serving the same purpose as longitudinal muscles.

Certain features about the digestive tract as a whole are worth noting. We have here to do evidently with a very simple structure and plainly derived from the proctodæum, stomodæum and mesenteron of the embryo, and but little modified therefrom. The three parts are separated by simple valves; there are no diverticula of either portion, and the histological structure corresponds, the epithelium, tunica propria, circular muscles, and connective tissue membrane being present in all three portions. The chitinous cuticle of the fore and hind guts is replaced in the mid-gut by the "Härchensaum," and the longitudinal muscles of the mid-gut are in the fore and hind guts replaced by dilator muscles passing outward to the body wall.

Fat Body.

Occupying a large portion of the body cavity is a mass of tissue which constitutes the "Exkretionsorgane" of Sommer, and which I regard as corresponding to the fat body of higher insects.

In the first abdominal segment it consists of a large mass lying on each side of the gut, and pressing against it, but having no prolongations to the outer surface of the body. In the metathorax occurs a prolongation to the hypodermis, just ventral to the legs. Farther forward this attachment becomes lost, and anterior to the metathoracic nerve ganglion a lateral one appears. This also soon ends, and in the region of the mesothoracic ganglion an attachment just external to the legs is formed. In front of this ganglion, this in its turn becomes lost and the lateral attachment reappears. At the prothoracic ganglion it again forms a prolongation to the body wall just external to the legs, while the two portions which press against the sides of the gut unite and surround both it and the heart, which has now bent ven-

trally into close proximity to the gut. In the back of the head this portion extends to the dorsal hypodermis to which it attaches, losing its other attachments. A little behind the brain it splits into two bands, one dorsal to the other. The dorsal portion passes forward, becoming reduced in size, and disappears just behind the brain. The ventral band splits into a right and a left half, and these diverge, passing around the sides of the brain, just anterior to which they end.

In the abdomen the limits of the fat body are more indefinite and it seems to occupy the greater part of the body cavity, attaching itself at many points to the hypodermis.

Similar masses of tissue occur in various portions of the body. Two particularly noticeable masses lie, one on each side of the ventral nerve cord, in the thorax. In many places the hypodermis is lined internally by a narrow strip of this tissue.

When examined with the microscope the fat body is seen to consist of a reticular network of fibers inclosing many granular portions, and containing many large deeply staining nuclei (Fig. 7). Its limits are sharply defined by an external membrane directly continuous with the basement membrane of the hypodermis when the fat body is attached to the body wall.

Sommer ('85), describing a similar structure in Tomocerus, states that it contains large numbers of concretions of a dirty white color in direct light, and pale green in reflected light, and which appear to consist of calcic carbonate ($CaCO_3$). In my specimens, with two exceptions, I have failed to find any trace of these concretions. These two exceptions, however, show, imbedded in the reticular tissue, what appear to be large granular irregularly shaped masses, of a dirty yellow color. More than this I have failed to discover. From their entire absence in most examples I conclude that they are transient formations, or else that they were entirely dissolved by the action of the acids used in the processes of preparation, except in the two cases mentioned. In those cases the granular masses seemed unaffected by the stains, as one was stained with Böhmer's Hæmatoxylin, and the other with Grenacher's Borax-carmine, yet the color of the concretions was alike in both.

Circulatory System.

The circulatory system consists of a dorsally placed heart, anteriorly continued as an aorta, and of the body cavity. These contain the plasma and corpuscles constituting the blood.

The heart lies on the median dorsal line just beneath the hypodermis, and ends posteriorly just above the junction of the mid-gut and hind-gut, at the pyloric valve (Fig. 18).

From its tip, which is slightly rounded, a thread of connective tissue runs back a short distance and attaches to the dorsal hypodermis on the mid-line.

From its hinder end the heart passes forward along the dorsal wall (Fig. 20) to the region of the fore-gut. Here it bends down towards the gut, to which it becomes attached by connective tissue fibers (Fig. 21). At the cardiac valve its structure is unchanged, but it becomes slightly smaller, and there being no ostia anterior to this place it is now termed the aorta. This rests directly upon the dorsal wall of the fore-gut. Passing forward we find the relations of the two changing; at the front portion of the prothorax (Fig. 23) it becomes flattened, and at the back of the head (Fig. 25) more than half encircles the œsophagus. A little farther forward it entirely surrounds the gut, the walls of which are bathed on all sides with blood, that portion of the aortal wall which was reflected around the gut apparently disappearing or else forming a part of the external limiting membrane (Fig. 26). Just behind the brain (Fig. 27) the aorta, while still encircling the œsophagus, stretches downwards till its ventral wall comes in contact with the dorsal surface of the sub-œsophageal ganglion. Beyond this point I have been unable to follow the vessel definitely, but from a study of a number of specimens I am inclined to think that it breaks up into a large number of small vessels, so short as hardly to deserve the name, which open directly into the body cavity.

The heart is held in place by five pairs of alary muscles, placed between the second and third thoracic, third thoracic and first abdominal, first and second, second and third, and third and fourth abdominal segments.

These muscles pass laterally to the dorsal wall on each side, where they attach themselves to the hypodermis between the dorsal and dorso-lateral bands of longitudinal muscles of the

body wall. They are triangular in outline, broadening as they approach the heart, near which they form two layers, one passing to the dorsal surface and the other to the ventral surface and ramifying there (Fig. 19).

In the space thus left between these two layers the heart wall is bent inward, and in the hollow thus formed is a small ostium opening into the cavity of the heart. The ostia appear to be in direct communication with the body cavity, as is the anterior end of the aorta, and the greatest portion of the circulation seems to be accomplished in the spaces between the fat body and the body wall. Valves were not observed in the ostia of the heart, but are probably present.

The walls of the heart have an outer muscular coating, and in some cases indications of a lining endothelium were observed.

Blood corpuscles occur in all parts of the circulatory vessels and in the body cavity. They are circular in outline and have a prominent nucleus. Their number appears to be very great.

Where the blood is aerated is uncertain, as there is no tracheal system present, but this question will be considered later.

Nervous System.

The nervous system consists of a supra-œsophageal ganglion or brain, joined by a double commissure enclosing the œsophagus to a sub-œsophageal ganglion, following which and joined to it and to each other by double commissures are three ganglia, situated in the prothorax, mesothorax and metathorax respectively.

Nicolet ('47) states that in the Collembola there are three ventral ganglia, two thoracic, and one abdominal one related to the reproductive functions; but he is clearly in error. Sommer ('85), speaking of Tomocerus, says that it has a brain, a sub-œsophageal ganglion, and four ventral nerve knots, three thoracic and one abdominal. Lubbock ('73) says: "The nervous system of Tomocerus consists of five ganglia, two of which, the supra- and infra-œsophageal, lie in the head. . . . The linear species have generally two ganglia in the thorax and one in the abdomen." If these observations can be given any value it indicates that the number of ganglia varies in different species and that no general statement for the group can be made.

The brain of Anurida is rather oval in outline when viewed from above, its long axis transverse to the body of the animal. It rests closely upon the fore-gut, over the sides of which it is continued down and more than half surrounds (Figs. 35, 36). A stout process leads forward on each side from the lower part of the anterior face, which becomes prolonged as the antennal nerve (Fig. 37). Farther back the dorso-lateral portion of each side becomes partially constricted off, forming the optic lobe, sending out a stout optic nerve, which almost immediately divides into five branches passing to the five ocelli of that side. These branches are termed the ocellar nerves (Fig. 35). In this same vertical plane also the ventral prolongations of the brain around the œsophagus form a trunk on each side, which passes downward and joins the sub-œsophageal ganglion, thus forming the circum-œsophageal commissure (Fig. 3). Posterior to this plane the brain abruptly terminates.

The sub-œsophageal ganglion is rather long and narrow, and is roughly cylindrical in form. From its anterior end a pair of stout nerves pass forward to the buccal cavity (Fig. 3, *b n*). A little behind this, on each side, it gives off from its dorsal angle a large nerve which passes back, branching as it goes, to the posterior extremity of the buccal pouch of that side. This nerve contains a large number of nuclei in a row along its axis. Just below this a smaller nerve is also given off from the ganglion. At its posterior end the ganglion is continued into two large lateral commissures and a small median one, all three leading directly backward to the prothoracic ganglion, the two lateral commissures each sending off a small trunk about half way between the two ganglia.

The prothoracic ganglion is shorter than the last; from near its middle it gives off a large trunk to the outside of the prothoracic leg on each side, and just posterior and ventral to this, another branch, to the inside of the leg.

Between this and the mesothoracic ganglion there is only the lateral pair of commissures, each commissure giving off a single branch.

The mesothoracic ganglion is like the prothoracic ganglion in form. It gives off from near its middle a single nerve trunk on each side, and it is joined to the metathoracic ganglion by a pair of commissures.

The metathoracic ganglion (Figs. 39, 40) appears to result from the fusion of two. About one-third of its length from its anterior end it gives off a pair of large nerves which almost immediately branch. Near its posterior end it gives off a pair of small nerves, and at its posterior end it gives off two large trunks which pass backward along the ventral side of the abdomen (Figs. 34, 45, *v. n.*) to the fifth segment. Here they break up, the largest branches passing to the genital glands close to where these enter the genital ducts. Between the metathoracic ganglion and the fifth segment they give off a number of branches to the different regions of the abdomen.

In the feet, especially in the two most distal joints, the nerve fibers enter small ganglia, each consisting of two or three cells (Fig. 38). In these cells are very large nuclei which stain deeply.

The histology of the nervous system offers little of interest. The brain and other ganglia have an external layer of small ganglion cells the walls of which are unstained by hæmatoxylin, while the nuclei stain deeply (Figs. 35–39). These cells seem to be aggregated into lobes in a slight degree. Besides the ganglion cells there is a central portion which does not stain, and shows traces of nerve fibrils passing in various directions. Over the surface of the brain and ventral ganglia is a tough dense layer of connective tissue.

In the ventral ganglia the unstained fibrillar mass is in two bundles, surrounded by ganglion cells (Fig. 39), and these bundles appear to be directly continuous with the commissures between the ganglia.

Near where each nerve trunk leaves the central nervous system, a large nucleus with several nucleoli (Figs. 37, 39) is observed. These may lie just where the nerve enters the ganglion (Fig. 37), or in the nerve itself (Fig. 39), and in the latter case they may be near the ganglion or at some distance from it.

That these large ganglion cells—for such they must be—are in some way related to the functions of these nerves seems evident, but their size as compared with that of the ganglion cells of the ganglia is so great that I am at a loss for a definite explanation, and I can find no description of any such nerve cells having been observed in any Arthropod.

The two abdominal trunks appear to contain no ganglion cells anywhere in their course.

Sense Organs.

These organs are quite well developed and are of great interest, as they have been but little studied, and the functions of some are wholly unknown.

Tactile Organs.—Long bristles plentifully distributed at intervals over the entire surface of the body have every indication of being tactile organs. They are especially abundant around the mouth and on the antennæ, where they form a dense clump at the tip (Fig. 46). A fortunate longitudinal section of an antenna (Fig. 47) shows at the base of a bristle a small bit of nervous tissue from which one nerve fiber passes up into the bristle, while another passes out to join the antennal nerve. Some of these bristles may perhaps have other than tactile functions.

A problematical organ, first discovered by Kingsley ('84) in Campodea, is present here, but in a more complicated form. As described for Campodea it is a small bilobed organ near the tip of the antenna. No description of its histology was given and its function is unknown.

In Anurida it occurs on the upper, inner side of the antenna, near the tip of the terminal joint (Figs. 46, 48). It is trilobed and its base rests in a slight depression of the cuticula. In section (Fig. 47) we see that the lobes are only separated in their outer halves, uniting in the half nearest the point of attachment. The cuticula covering the organ is smooth and follows the surface of the hypodermis which lies immediately beneath. A good sized nerve leads to the organ (Fig. 47).

Its function can at present be only surmised, but the three lobes are so placed relative to each other that they might easily give the animal some idea of the form of any object, corresponding as they do to the three dimensions of space. Some species of Collembola are wholly blind, while others have only such simple eyes that their vision must be at best extremely poor; and from these considerations and from the location of the organs in question I am inclined to regard them as organs of form supplementing the eyes and tactile bristles.

Eyes.—Compound eyes do not occur in any of the Collembola,

and "the number and arrangement of the ocelli afford good specific and generic characters."

Templetonia has one, Orchesella six, Tomocerus seven and Smynthurus eight ocelli on each side. They are usually placed in a mass of pigment, denser than elsewhere.

In Anurida there are five ocelli on each side (Fig. 41). Viewed from the surface they appear as small round or slightly oval convex bodies, the cuticula of which is smooth. Vertical sections (Fig. 42) show that each consists of a nearly spherical mass of protoplasm lying beneath the corneal cuticula, and containing four nuclei which show several plainly marked chromatin granules. Immediately beneath the protoplasm is a dense mass of pigment, which between the ocelli completely fills the hypodermic cells. What relation such an eye as this has to an ommatideum is uncertain, but its structure might be explained by assuming the fusion of four cells of the hypodermis, their cell walls becoming lost and the outer surface becoming convex, while the pigment passes to a deeper layer and a smooth corneal cuticula is formed.

Post-antennal Organ.—This curious structure (Fig. 41, *p. a. o.*) was discovered by Laboulbéne, who proposed to call it the "organ prostemmatique ou ante-oculaire." Tullberg ('71), however, objected to this name, as a corresponding structure is present in some species which have no ocelli, and he therefore suggested the term "post-antennal," which has been generally adopted.

Laboulbéne describes this organ as follows: "Cet organ est formé par des espaces colorés tels que les représente la figure 7; leur couleur est très-noire. Le nombre des cercles rapprochés varie de 7 à 8, le plus ordinairement il y en a 7, mais je dois noter que j'en ai trouvé parfois 8 d'un côté et 7 de l'autre. Sur les jeunes individus, la disposition est très curieuse, la figure 9 en donne une idée: il existe alors 22 à 24 espaces comprimés et serrés les uns contre les autres avec une espace central libre: le tout rappelle la form du fruit chez les plantes malvacées indigènes entre autres les *Malva* et les *Althaea*. Quelque soin que j'aie mis à chercher si du point central il naissait un poil allongé ou toute autre production dermique, je dois dire que je n'en ai point trouvé."

In the examples I have examined the organ consists of an

irregular circle of oval bodies with an external cuticular covering, and an internal portion filled with black pigment and connected with the hypodermis beneath. The number of oval bodies varies from seven to nine, and like Laboulbéne I have observed a few specimens with seven on one side and eight on the other. The usual number, however, is seven.

These organs occupy a slight depression on each side of the head between the antenna and ocelli. Near the organ the elevations of the cuticula cease, and over the surface of the organ itself it lies smoothly (Fig. 43).

Focusing more deeply than is represented in Figs. 41 and 44, a faint circle appears at the inner end of each oval body. Vertical sections (Fig. 43) show this circle to be the outline of a pedicle or stalk on which each body is mounted, and through the center of which, connection between the pigmented protoplasm occupying the centers of these bodies (removed in Fig. 43) and the protoplasm of the hypodermis, is maintained.

The function of this organ is unknown. I have endeavored to find some nerve connection with the brain, but on account of the amount of pigment surrounding it I have been unsuccessful. In one instance I thought I discovered a nerve leading from the brain just anterior to the optic lobe, to it; but while such a nerve from the brain certainly passes in that direction, it enters the pigment mass a little to one side, and beyond that point I have failed to follow it.

One is naturally inclined to consider it as in some way possessing a visual power, but there is no evidence supporting this view, and the structure does not materially aid in a decision.

Abdominal Vesicle.

The abdominal vesicle or ventral tube of the Collembola is an organ of great interest. Its form and relations differ greatly in different species, and a description of its comparative structure in different forms, and a discussion as to its functions, is reserved for later consideration.

In Anurida maritima it is a large protuberance on the ventral side of the first abdominal segment. It is divided into halves by a median longitudinal cleft, so that it may be considered either a bilobed structure or as formed by the pressing together

of two separate protuberances. Cross sections of the animal in this segment show that the cuticula follows all the folds of the surface (Fig. 34. The section knife has here separated the cuticula and hypodermis somewhat). Beneath the cuticula on either side of the vesicle the hypodermis shows its normal structure, and this is unchanged on the lateral sides of the vesicle itself. At the tip of each lobe, however, its character changes, being modified to form a layer of large granular gland cells with large nucleolated nuclei. These cells lie beneath the cuticula of the longitudinal cleft, in which the usual small protuberances of the cuticula are absent. Several muscles pass from the body wall into these lobes, which may be thus retracted in some measure.

Leading forward from the abdominal vesicle on the median line is a peculiar structure somewhat tubular in appearance, shown in section in the mesothorax in Fig. 33, and under the head in Fig. 32. This tube originates at the base of the median cleft at the anterior end of the vesicle, and is formed by a pair of outgrowing ridges of the hypodermis, which pass outward from the surface of the body, then towards each other, then towards the body again till they touch it, thus forming a pair of tubes with a groove separating them. In both figures the cuticula and hypodermis have been torn apart, and the former has preserved most nearly the uninjured structure of the tubes.

Farther forward the two outgrowths, instead of returning to the body, fuse together, and in this way form a single tube (Fig. 32).

In the postero-lateral and dorsal regions of the head is a mass of cells which I at first supposed to be the salivary glands (Fig. 29). Their histological appearance is that of the salivary glands, and on each side of the head a convoluted duct (Fig. 30) leads downward, then forward to the buccal cavity. Here it is joined by the duct from the other side of the head, and the median duct thus formed passes forward to the posterior end of the median cleft of the mouth, gaining a chitinous lining. Here, to my surprise, I found that instead of bending upward to enter the buccal cavity, it bent downward, passing through the ventral cleft of the mouth (Fig. 31), and communicated directly with the tube leading forward from the abdominal vesicle.

I long doubted whether I was not mistaken in my observations, but a careful study with high powers of about twenty-five specimens only confirmed my previous work, and I am therefore compelled to think that though occupying the position of salivary glands, and with ducts taking the usual course of salivary ducts, these glands must have some other function. It is possible that they may have a connection with the buccal cavity also, by some branch from the main duct, but I have failed to find any such arrangement.

The histology of the gland and of the ducts is like that of the salivary glands and ducts of the higher insects. The cells of the gland (Fig. 29) are granular, with prominent nuclei; those of the duct are cubical, with indistinct boundaries (Fig. 30) and with smaller nuclei.

Ventral Spring.

In young examples of this species Ryder ('86) describes a rudimentary pair of processes on the ventral surface pointing forward from the anterior edge of the fourth abdominal segment. This structure he regards as a rudimentary spring, and says that no trace of it occurs in the adult. In the same place I found in sections two nearly spherical masses of cells, slightly resembling gland cells in a stage of degeneration, separated from the hypodermis by the fat body, which here lines the body wall (Fig. 45).

What these masses represent I do not know, unless they are the rudiments of the structure described by Ryder in a stage of degeneration, remaining after all external trace of the structure is lost. If it is indeed the rudiment of a spring, it is of much interest as indicating that the differences between the springless Collembola and those which possess a developed spring are due to the degeneration of the former group.

Genital Organs.

The genital organs lie ventro-lateral to the abdominal portion of the digestive tract, but may reach forward into the mesothorax. Both the male and the female organs consist of a pair of simple tubes of varying diameter, containing the reproductive elements. At their anterior ends these tubes are attached by a cord of connective tissue which passes forward and joins the

ventral body wall. Posteriorly each tube opens into a duct, which soon unites with that of the other side, forming a single median duct leading to the exterior. In the female a ventral diverticulum from this portion of the duct is also present. The genital opening is situated at the tip of a small papilla on the hinder edge of the fifth abdominal segment on the median line of the ventral surface of the body.

Female Genital Organs.

The two ovarian tubes lie generally ventro-lateral to the gut, but when distended with eggs may be pressed dorsally till portions may become dorsal to it. Externally they are covered by a sheet of dense connective tissue, within which lie cells of various sizes, containing very large nuclei and a large number of yolk spheres.

The anterior end of the ovary (Fig. 56) is drawn out into a narrow tube, which is filled with granular protoplasm and small oval nuclei containing several distinct nucleoli. Passing down the tube the nuclei become more spherical and pass to one side, along the wall of which they form a sort of germinal ridge. The nuclei increase rapidly in size and gather the protoplasm about them, forming cells (Fig. 57). These cells are pushed out from the germinal ridge by cells forming in a similar way behind them, and these are in their turn pushed out by others, and in this way cell rows are formed, which coil about the walls of the ovary and across it (Fig. 55).

Soon a sort of degradation occurs, many of the cells of each row becoming completely destroyed. In this change the nuclear chromatin leads the way, forming irregular scattered granules, and this change continues till of an entire row of cells but one or two remain intact. Whether but one cell of each row becomes an ovum, or more than one, I do not know.

The broken-down matter assumes the form of globules of different sizes, at first small, but rapidly enlarging, and gradually becomes the yolk, which is very abundant in each ovum.

As the ova pass backward along the ovarian tubes they in some way acquire an inclosing membrane, but how and just where I cannot say. Even near the opening of the oviduct each ovum has several cells of the row from which it was formed still intact,

and the final breaking down of these cells must occur very late in the maturation of the ovum.

Passing from the ovary is a narrow oviduct (Fig. 53) consisting of granular cuboidal epithelial cells, resting on a delicate tunica propria and containing small spherical nuclei.

The oviducts of the two sides soon unite and the two together form the vagina, which has a wall of large columnar cells, broader on their attached edges than at the lumen. The nuclei of these cells are large, round, and contain prominent nucleoli. The cells rest upon a tunica propria continuous with that of the oviducts, and bear on their faces which form the lumen, a chitinous cuticle (Fig. 54).

From the ventral side of the vagina a small diverticulum passes forward (Figs. 53, 54) which may possibly be a receptaculum seminis, and which is slightly bilobed at its anterior end.

The cells of this diverticulum are quite granular and the cell walls are less distinct than those of the vagina, and fewer nuclei are present. Externally the cells rest upon the tunica propria, and internally they are lined by a continuation of the cuticle of the vagina, which here bears relatively long spines projecting into the lumen of the diverticulum.

No circular muscles are present around these ducts, but dilator muscles (dilatores vaginæ) are present leading to the body wall (Fig. 53).

At the genital opening the cuticle of the ducts becomes continuous with the cuticula of the external surface of the body.

Male Genital Organs.

The testes, like the ovaries, are a pair of long tubes extending backward from the mesothorax. They differ greatly in their histological structure at different stages of maturity. The immature testis is shown in cross section in Fig. 49. It is surrounded by a dense layer of connective tissue, and consists of a mass of protoplasm containing granules and scattered nuclei. The granules stain like chromatin granules and form groups surrounded by protoplasm denser here than elsewhere. At this age the whole testis has the same structure. Farther development begins at the posterior end, and passing forward from that point, successively earlier stages of development are met with.

If we examine a mature testis we find at the anterior end the condition shown in Fig. 49. Farther back the structure changes to that shown in Fig. 59, the groups becoming more concentrated and septa appearing. A little behind this point the granules elongate, and from them pass fine fibers or threads which form bundles lying in various directions (Fig. 50). The nuclei are still present, scattered through the substance of the testis, and at one point a structure slightly resembling a germinal ridge occurs. The appearance of this part of the testis is also shown in longitudinal section in Fig. 58, near the line *AB*.

Behind this the structure soon changes. The entire substance begins to undergo degeneration: the threads become lost, the septa disappear and we get a condition shown in Fig. 60 and along the line *CD* of Fig. 58. Soon this mass becomes more densely granular: fat globules now appear in small numbers (Fig. 61 and along the line *EF*, Fig. 58), and soon a columnar epithelium appears to be formed from that portion nearest the external connective tissue. This epithelium (Fig. 62) has large nuclei and indistinct cell boundaries. A somewhat similar appearance of that portion of the granular mass nearer the center of the tube may also be observed (Fig. 58), forming a zone separating the unchanged mass (along the line *EF*, Fig. 58) from the mass of fat globules and fine particles which follows. The line *GH* of Fig. 58 crosses the mass of fat globules and also a portion of the separating zone. Passing back, the fat globules increase in number and nearly fill the posterior part of the testis, while among them are multitudes of spermatozoa with elongated, deeply staining heads.

In brief, the process of spermatogenesis is as follows: The granular groups (Fig. 49) become greatly developed and form distinct groups containing elongated granules (Fig. 50). This structure is soon broken down and a quite homogeneous mass results, containing many deeply staining granules (Fig. 60). The granules soon become more pronounced, while the remainder of the substance present collects around the scattered nuclei, forming epithelial cells. In these cells, or by modifications of them, the fat globules and spermatozoa are formed and make their way into the lumen of the testis.

The testis opens into a short vas deferens consisting of low

cuboidal epithelial cells resting on a tunica propria. The vasa deferentia soon unite and form an ejaculatory duct (Figs. 51, 52, 63) lined by a chitinous cuticle continuous with the cuticula of the outer surface of the body. In some specimens (Fig. 63) this is thrown into ridges on the ventral wall of the duct, but in other cases (Fig. 51) these appeared to be entirely absent.

Beneath the cuticle are large, granular, columnar epithelial cells with distinctly staining nuclei. At the external opening of the ejaculatory duct these cells bend each way and are continued along the surface, retaining their columnar character; for some distance before assuming the normal structure of the hypodermic cells (Figs. 51, 63) these cells rest upon a tunica propria continuous with that of the vasa deferentia. No circular muscles occur, but dilator muscles (Fig. 63), running to various parts of the body wall and serving by their contraction to enlarge the ducts, are met with.

Molting.

The process of molting is much the same as in other Arthropods. The external cuticula, the cuticle of the fore gut, hind-gut and of the genital ducts, is shed with each molt. In the mid-gut, just before ecdysis, a peculiar change occurs. The nuclei of the epithelium divide and one of the two that are thus formed in each cell passes towards the free face of the cell, while the other passes towards its base. The cell walls now become indistinct and delamination occurs, the outer half of each cell being thrown off. These molted cells collect in the lumen of the gut and remain there (Figs. 14, 15) till the chitin of the remainder of the body is thrown off, when this is also removed from the body. The outer edges of the cells remaining form a new "Härchensaum" and resume their normal condition.

General Considerations.

Two problems in connection with the anatomy of the Collembola require further consideration. These are: 1. The relations of the fat body to the hypodermis, and an examination of the method of formation of the body cavity. 2. The function of the abdominal vesicle and of the glands in the head to which it is joined by the small ventral tube, and the homologies of these parts.

The fat body of the higher insects is regarded as of mesoblastic origin, and any protoplasmic relations it may acquire with the hypodermis must therefore be of a secondary nature. In Tomocerus, Sommer describes such a relation, and from this decides that the structure is epiblastic, and not homologous with the fat body of other insects.

If we consider the method of formation of the body cavity in Arthropods as described by Sedgwick ('87) for Peripatus, we find that in the solid mesoblast a series of splits appear, enlarge, fuse and form cavities of varying form and size, constituting a body cavity of the type termed by Lankester a hæmocœl.

As the mesoblast on the one side lies closely against the hypoblastic mesenteron, and on the other against the epiblast which is to form the hypodermis, the relations of the fat body to these may be explained as follows:

The layer of mesoblast cells next to the splits becomes flattened to form a pavement epithelium which lines the body cavity. Where the mesoblast is several layers of cells in thickness, the middle layers do not become flattened but form the mass of the fat body of the adult. Where the body cavity is so large that but a single layer of mesoblast cells remains between it and the hypodermis, this layer, as elsewhere, becomes endothelial and forms the tunica propria upon which the hypodermic cells rest. In connection with the gut it forms the external limiting membrane, which is lacking where the unmodified cells of the fat body and of the gut meet. At these places, however, there seems to be a sort of membrane between the muscles of the gut (which are themselves of mesoblastic origin) and the fat body. This membrane may perhaps be derived from a flattening of the layer of cells of the fat body which is nearest to the muscle layer, and would correspond to the limiting membrane of the hæmocœl, and it is possible that a membrane similarly formed between the hypodermis and the fat body marks the distinction between the two, seen in the higher insects. Strings of mesoblast cells passing through the body cavity or along its walls become converted into muscle fibers. The fat body is so universally present in insects, Myriapods and in Peripatus, that it must be looked upon as a very primitive structure, and, as already stated, appears to be mesoblastic in origin.

I have failed to observe any protoplasmic connection between the fat body and the hypodermic cells in Anurida, but if Sommer's observations on Tomocerus are correct, then, notwithstanding the facts just mentioned, we must either regard this structure, corresponding in position to the fat body, and which, except in its relations to the hypodermis, might be regarded without difficulty as mesoblastic, as in no way homologous with the fat body, or assume that the external cells have secondarily acquired a connection with the hypodermis. Sommer declares in favor of the first of these views and decides that this structure is not homologous with the fat body. My own inclination is to accept the other view and question the actual existence of protoplasmic connection between the cells of the fat body and of the hypodermis.

The abdominal vesicle has been differently interpreted by different writers. By Latreille and Kolenati it was regarded as a genital organ: Bourlet considered it as adhesive; an aid in easing the shock of alighting after a jump, and as serving to hold and to moisten the spring. Nicolet, Lubbock and Tullberg consider it an adhesive organ. Reuter says that water is gathered to the mouth from the surface of the body by the tarsal claws, from which it is placed by the "Haftlappen" in the interior of the tube. Sommer believes it to be adhesive and suggests that it may possibly have a function similar to that of the oil glands in birds.

It differs in structure in different genera. In Podura, Lipura and Anurida it is a simple cleft tubercle. In Tomocerus it is enlarged, forming a tube, bilobed at the tip. In Smynthurus it has a similar structure, but "from the end of the tube the animal can project two long delicate tubes provided at their extremity with numerous glands." No one seems to have observed the connection of the little tube leading forward, with the vesicle and with the glands in the head.

Meinert, describing this little tube, writes as follows: "As yet no spiracles have been observed in Poduræ; but, according to my observations, their organs of respiration consist merely of an open canal along the underside of the head and the thorax, and it is in the fore end of this canal, under the head, that the tracheal system opens, whenever it exists, as in some of the larger Smynthuri."

This view seems to be unsatisfactory, as opportunities for the purification of the blood are in no way bettered by the form or position of the tube, while its connection with the glands of the head seems to indicate that its function must be other than respiratory.

As regards the vesicle, the observations of Lubbock on Smynthurus would appear to be quite conclusive. He says: "If a Smynthurus is laid on its back—a position from which it has some difficulty in recovering its feet—and if while in this attitude a piece of glass is brought within its reach, the animal will endeavor to seize it with the feet, but at the same time it will project one or both of the ventral tentacles and apply it, or them, firmly to the glass, emitting at the same time a drop of fluid, which, no doubt, gives a better hold."

The comparative studies of Haase ('89) on the ventral sacs of Scolopendrella, Diplopoda, Thysanura and the abdominal vesicle of Collembola, lead him to consider these all as homologous structures. His views as to the structure and functions of the abdominal vesicle I give in his own words, taken from his summary.

"7. Der Ventraltubus der Collembola am ersten Abdominalsegment besitzt ebenfalls entwickelte Rückziehmuskeln, einen durchgehenden bindegewebigen Strang, im Innern einzelne mit deutlichen Poren ausmündende, ein Sekret aussondernde Drüsenzellen und tritt durch einströmen von Blut hervor.

"8. Nach Beobachtungen an lebenden Stücken von Machilis und Poduren treten die Abdominalsäcke nur bei vollkommener Ruhe des Thieres und in feuchtwarmer Luft hervor, bei Poduren liess sich unter dem Mikroskop eine bedeutende und schnelle Durchströmung des ausgestreckten Ventraltubus durch Blutflüssigkeit in der Richtung nach dem Vorderrande des Körpers zu, verfolgen.

"9. Beobachtungen über das Kriechen der Poduren an Glaswänden zeigen dass der Ventraltubus dabei oft unthätig ist wie die Abdominalsäckchen in gleichem Falle stets bei Machilis.

"10. Die Beziehung der Ausbildung des Tracheensystems zu der der Ventralsäckchen ergeben ebenfalls, dass letztere eine respiratorische Funktion haben und als Blutkiemen anzusehen sind; die Luftröhren fehlen bei den meisten Poduriden, sind

verkümmert und öffnen sich durch ein Stigmenpaar bei Smynthurus und Scolopendrella, durch drei bei Campodea; das Tracheensystem ist in Rückbildung begriffen bei Lysiopetalum, Polyzonium und ausserdem erst wenig entwickelt bei Machilis; mit der Ausbildung der gemeinsamen Längsstämme erfolgt die Rückbildung der Ventralsäckchen." Elsewhere he states that a slight glandular function for the purpose of adhesion, as described by Lubbock, has probably been recently acquired.

This explanation certainly has much in its favor, but leaves unexplained the function of the little tube and the glands in the head connecting with it. It may be that the relation of these parts in Anurida is a secondary one: indeed, it is evident that the glands in the head are homologous with salivary glands and their unusual relations are as yet unexplained. Hence we must consider this problem as at best but partly solved.

Notes on Lepisma saccharina, Linn.

This little animal is a pest to housekeepers, "eating cloths, tapestry, silken trimmings of furniture, and doing occasional damage to libraries by devouring the paste and eating holes in the leaves and covers of books." It is from 6 mm. to 9 mm. long, of a silvery white color, and with the division into head, thorax and abdomen distinctly marked.

The head is rounded and bears a pair of filiform, many-jointed antennæ, rather more than half as long as the body. Posterior to the bases of these are the eyes—a mass of twelve facets on each side of the head.

The mouth is distinctly ventral in position and differs from that of Anurida in having well-developed external mouth-parts—labrum, mandibles, maxillæ and labium—which will be described later.

The thorax is convex above, flattened beneath, and with laterally produced terga. It has three pairs of well-developed legs, the tarsus of each ending in a pair of claws.

The abdomen is tapering, cylindrical, and consists of ten segments, the second and ninth being rather short. From the terminal segment three long, many-jointed stylets (cerci) are given off.

In the female an ovipositor is present which the drawing given

by Grassi ('87*a*) (Fig. 64) indicates as being composed of a pair of processes from the seventh sternote and a pair from the eighth. The figure also shows on each of these segments and that which follows, a pair of lateral processes—" Pseudo-zampe." Haase ('89) differs from Grassi as to these appendages which he terms " Abdominalgriffel." He says: " Bei Lep. sacharinum kommen nur an den zwei vorletzten Hinterleibsringen Abdominalgriffel vor, und zwar sind dieselben am achten Segment 0.6, am neuntem aber 0.8 mm. lang." Thus he finds but two pairs instead of three. He says that in a Trieste species the ovipositor arises as in Machilis, which he describes thus: " Sie [the Gonapophyses] bestehen bei den Weibchen aus vier, die Legescheide bildenden Ventralanhängen des achten und neunten Abdominalringes und umgeben die am Ende des achten oder eher, wie es die Regel scheint, am Anfang des neunten Segments ausmündende Geschlechtsöffnung."

The surface of the body is covered by scales resembling those of the wings of butterflies, and in many places occur groups of bristle-like hairs which probably have a sensory function.

I am not quite certain as to the species which I have studied, as while it agrees fairly well with the descriptions of Lepisma saccharina in other regards, instead of its scales having "from twenty to twenty-six" ribs (Beck; Lubbock '73), they have nearly twice that number.

The specimens were treated as were those of Anurida, except that no ether was added to the killing fluids.

Integument.

The integument, as in Anurida, consists of a hypodermis resting upon a basement membrane and covered by a chitinous cuticula. The hypodermis contains no pigment, and consists of a single layer of cells with large, closely packed nuclei (Fig. 66) and with the cell boundaries not brought out by the stains used. The cuticula is not covered with protuberances as in Anurida, but is smooth and is perforated by the pedicels of the scales. These (Fig. 65) vary somewhat in form and size. They have parallel longitudinal ribs, between 40 and 50 in number, and a short pedicel which penetrates the cuticula nearly to the hypodermis. The scales are attached at their anterior ends and their

free edges overlap the scales next posterior to them. It is these scales which give the silvery gloss to the living animal.

The hairs which are present on some portions appear to have some connection with the hypodermis (Fig. 78), as the entire thickness of the chitin at their places of attachment appears somewhat modified.

On the dorsal surface of the thorax the cuticula is much modified at the sutures between the segments (Fig. 67), and at these places muscles are frequently attached. At various places on the body the integument seems to have been folded in on itself to form processes for the attachment for muscles. One such is very noticeable and is located on the anterior face of the head, which it partially crosses in a horizontal direction.

Muscular System.

Owing to the difference in the mouth-parts the muscles of the head are more developed in Lepisma than in Anurida. Other differences in distribution also occur, but I have not examined them. Histologically, there is little worthy of note, except that in some fibers Krause's membrane is remarkably distinct, and that nuclei which lie at or near the centers of the fibrils (Fig. 68) are present in great abundance. There is a greater condensation of the fibrils into bundles than in Anurida. The attachment of the muscles to the cuticula appears to be the same as already described in Anurida.

Digestive System.

The mouth is situated on the ventral side of the head and bounded anteriorly by the labrum, which is a broad, flattened portion of the ventral integument at that place. Close behind this, at the sides of the mouth-opening, are the mandibles (Fig. 69). These are stout chitinous jaws, each with several pointed teeth and a flat grinding surface, at the base of which is a cluster of short bristles. Muscles enter the mandibles in considerable numbers and pass in different directions. The maxillæ (Fig. 70) are smaller, and consist of a lacinia bearing two pointed teeth and a few scattered bristles, a rounded galea, and a palpus which projects at right angles to the maxilla and which consists of five quite long, cylindrical segments, covered with hairs.

The labium (Fig. 71), which forms the posterior limit of the mouth, consists of a doubly bifid lip and a pair of three-jointed palpi, with a small plate overlying the basal joint and which is usually regarded as a fourth segment, but which is considered by Grassi, whose figure I reproduce here, as not forming any part of the palpus proper. The terminal segment of the palpus is the largest, is flattened, and near its tip bears three (Grassi '87*a*) or five (Oudemans '87) sense organs.

Surrounded by these mouth-parts is the oral opening which leads into the pharynx. This passes dorsally and somewhat forward for a short distance, then turning posteriorly, passes beneath the brain, through the circum-œsophageal commissure and enters the thorax, gradually increasing in diameter and changing in its histological structure. This portion, which extends to about the end of the fourth abdominal segment, may, for convenience, be termed the œsophagus, though it passes insensibly into the pharynx at its anterior end.

The pharynx (Fig. 72) consists of four layers. Resting on a basement membrane is an epithelial layer which is thrown into very irregular and strongly marked folds. In this layer nuclei occur but no cell walls are apparent. Separating the epithelium from the lumen of the pharynx is a layer of chitin which follows every fold of the epithelium. External (toward the body cavity) to the basement membrane is a layer of circular striated muscles. These are closely packed, side by side, at the beginning of the pharynx, and show frequent nuclei which, unlike those in Anurida, have no definite location in the fiber. Farther from the mouth the fibers separate, leaving a little space between each two. No longitudinal muscles are present. External to all is a connective tissue sheath—the lining membrane of the body cavity. In the œsophagus the epithelial folds become smaller and finally disappear (Fig. 73).

Near the end of the fourth abdominal segment the œsophagus quite suddenly contracts, chiefly on its ventral and lateral portions, forming a tube less than half the size of the œsophagus. This tube, which is called by Rovelli the crop, has a slight ventral curvature. Near the end of the fifth segment six longitudinal chitinous bands appear in its walls, their bases interrupting and dividing into six parts the layer of circular muscles which is

here greatly developed. These bands project into the lumen of the tube and bear stout horny teeth, while the chitinous cuticle of the remainder of the crop bears slender backward-pointing spines. A layer of longitudinal muscles external to the circular layer arises on the œsophagus near the end of the third abdominal segment and passes back to this region, but its exact distribution I have failed to ascertain. Beyond the chitinous bands of teeth the tube turns dorsally, then bends suddenly towards the ventral side of the body and enters the mesenteric portion of the digestive tract—the stomach.

The stomach at its anterior end consists of six blind pouches, three on each side, which surround the crop and extend forward nearly to the end of the œsophagus. The ventral pouch on each side fuses with its fellow as it passes backward, and into this open the two others on each side and the crop on the median line. The stomach now passes back into the seventh segment where it abruptly turns to the left and then towards the head, forming a half circle, where it ends. The ileum which now follows gives off the Malpighian tubes and turns posteriorly, completing the circle and passing dorsally to the stomach. About one segment posterior to this point it enters the rectum, which is a large, irregularly shaped chamber with many folds and hollows. This finally narrows into a very short, straight tube which leads to the anus, and from the walls of which dilator muscles pass in different directions to the body wall.

The histological structure of these divisions remains to be described. The stomach (Fig. 74) has a well-developed layer of columnar epithelial cells with large spherical or slightly oval nuclei and indistinct cell walls. There are a large number of small crypts in the epithelium, which have a glandular appearance. Oudemans ('87), however, describing a similar condition in Machilis maritima, says that the young cells designed for regeneration, directly dividing, and as yet not secreting, lie in crypts which are not considered as glands. The ends of the epithelial cells bear what is probably a "Härchensaum," which has been already described for Anurida and which corresponds in appearance with that there present. The epithelium rests upon a basement membrane, external to which is a layer of circular striped muscles and a few scattered longitudinal ones, and finally a connective tissue membrane surrounding all.

The ileum (Fig. 75) has the same layers, the "Härchensaum" being replaced by a thin chitinous cuticle, showing this portion of the digestive tract to be of proctodæal origin. The epithelium is thrown into six distinct longitudinal folds and its cells have well-marked boundaries, seemingly continuous with processes from the basement membrane which follows these folds and is very noticeable in sections. The circular muscles do not follow the infoldings of this membrane but have spaces at those points. The ileum is held in place by mesenteries which pass to the body walls and which apparently include a striated muscle fiber continuous with the layer of circular muscles of the ileum.

The rectum (Fig. 76) has its epithelium thrown into many and pronounced folds. The cells are tall, columnar, with very faintly marked walls and with the nuclei near the bases of the cells. The protoplasm is homogeneous, stained only slightly by hæmatoxylin, and differs greatly in appearance from that of the stomach. The basement membrane follows all the folds of the epithelium, and striped muscle fibers ramify over it in all directions. The chitinous intima bears a few small short spines.

The salivary glands open into the pharynx just in front of the labium by a median duct which leads back close beneath the sub-œsophageal ganglion for a little distance. It then forks and forms the glandular portion of each side, which lies in the posterior lateral part of the head and is a simple tubular gland. The duct is lined by a chitinous cuticle.

The Malpighian tubes (Figs. 74, 77) are long, convoluted tubes which arise from the alimentary tract close to the junction of stomach and ileum. Their number is not determined. Ramdohr found two, Siebold and Gegenbauer find four, while Leuckart finds six and Schindler ('78) eight. Rovelli states that there are but four and Grassi ('87 *a*), in his diagram of the digestive system of Lepisma, represents but three. From what he says, however, I infer that he regards the number as variable. I have been unable to ascertain the number in the examples which I have examined, but feel certain that there are at least four.

The tubes each consist of a single layer of cells with the walls rounded externally and internally (Fig. 77), and none visible between neighboring cells. The nuclei are quite large, round

or oval, and, scattered through the protoplasm often, are yellowish, highly refracting granules which also occur in the lumen. The protoplasm of the cells stains quite deeply. Whether there is a chitinous intima lining the lumen or not, I cannot say.

Fat Body.

The histological appearance of the fat body is much the same in Lepisma as in Anurida. Its distribution is not so sharply defined, however, and can be best described by saying that it occupies almost the entire body cavity, while through it pass nerves, muscles, etc. Its relation to the hypodermis appears to be much as in Anurida, except that where the two are contiguous there may sometimes be observed what is apparently a delicate basement membrane separating them (Fig. 78).

Circulatory System.

The heart is situated as in Anurida on the dorsal median line and extends forward from that portion of the abdomen where the ileum bends downward to join the rectum, to the thorax. Here it very gradually becomes smaller and, as the aorta, continues to the prothorax, where it bends down and comes to lie close above the œsophagus. When it has attained a point dorsal to the sub-œsophageal ganglion it becomes lost. The histological structure of these portions agrees with that in Anurida.

The posterior end of the heart, instead of forming a rounded, closed end, becomes much reduced in size, the lumen being only large enough for one or two blood corpuscles to lie in side by side, while its walls become very muscular (Fig. 79). This portion of the dorsal vessel soon becomes attached to the dorsal hypodermis and finally, near the base of the median anal stylet, opens with a flaring mouth like that of a trumpet, into the body cavity. Near this point it is surrounded and firmly held in place by numerous bands of muscles leading from the dorsal body wall to a median diverticulum of the rectum lying just beneath. One or two of the more median of these muscles appear to bend around the ventral side of this blood-vessel and become attached to the hypodermis again, thus placing it in a sort of sling. Figs. 79 and 80 represent sections of this blood-vessel and of the heart

proper, drawn from the same series of sections and equally magnified.

I have met with no description of the posterior portion of the dorsal vessel which agrees with what I have just described, and long doubted the accuracy of my observations. The same thing occurring in all my specimens, however, and the confirmation of the above description by others who examined my sections, lead me to think that it is neither an individual variation nor a result of a too hasty examination, but a normal feature in the animal. As to its function I can only suggest that as it is highly muscular and near the posterior end of the body, it may be the chief contractile portion of the vessel.

The blood corpuscles (Fig. 80) apparently differ in no way from those of Anurida.

Tracheal System.

The tracheal system is present, but owing to lack of material for special examination on this point, I can say but little of it. The stigmata open into tracheæ which have a chitinous lining with delicate spiral threads. The tracheæ pass chiefly in a longitudinal direction and with but little branching. Almost any anterior section of the abdomen will show two trunks cut transversely, lying quite near the lateral surfaces of the body.

Nervous System.

The nervous system comprises a brain (supra-œsophageal ganglion) and a sub-œsophageal ganglion united by a circum-œsophageal commissure, three thoracic and eight abdominal ganglia. The two first-mentioned ganglia are situated in the head; the last thoracic and first two abdominal ganglia are fused together (Fig. 81), and the seventh and eighth abdominal ganglia are also fused (Fig. 82). From the brain the antennæ and eyes are innervated, and from the sub-œsophageal ganglion the different mouth-parts.

Histologically, the brain shows many features of resemblance to the brain of the cricket (Fig. 83), but I shall not attempt a description of its parts. No trace of giant cells near the origins of the different nerves, such as was observed in Anurida, was seen.

Sense Organs.

The only sense organs which I have observed are the tactile organs and the eyes.

Tactile organs.—These differ little from those of Anurida, except that at their bases there appears to be a distinct joint with the cuticula (Fig. 78), as is usually the case in higher insects.

The bristles are scattered over the entire body, but are especially abundant on the head. The long and slender antennæ and the three anal cerci which contain no muscles, have each a nerve fiber and are true tactile organs. No such trilobed organ as that described for Anurida was observed.

Eye.—The eye is not "an aggregation of ocelli," but is a true compound eye, directly comparable to that of other Arthropods and very different from that of Anurida. Each eye is composed of twelve ommatidea which form a cluster of a pyramidal shape, the consequence of the similar shape of each ommatideum (Fig. 84). The external facet—the cornea—of each ommatideum is large, and immediately beneath it are two large cells which constitute the corneagen. These cells are much thinner at the axis of the ommatideum than at the periphery, and are therefore somewhat triangular in section.

Next internal to the corneagen is the crystalline cone, which is a large concavo-convex mass of chitin nearly filling the entire ommatideum at that level. Just external to it and filling the space remaining at its sides are four small cells constituting the vitrella. Filling the inwardly concave portion of the crystalline cone and passing inward is a rather long, pyramidal chitinous mass—the rhabdomere. Immediately surrounding this and prolonged beyond its tip are four densely pigmented cells, forming the retinula. The prolongations of these cells perforate a basement membrane just internal to the group of ommatidea and become the optic nerves, passing to the brain. Surrounding each ommatideum and filling the spaces between neighboring ones is a mass of small pigment cells.

If the parts of an ommatideum be pushed away from the central axis in all directions, the outer layers moving most, while the retinulæ retain their natural position, a pit is formed, and if this arrangement be projected onto a plane the result is that shown in Fig. 85. In the drawing two of the retinulæ have the

pigment removed, and the chitinous portions of the two outer circles are represented as narrow bands and not of their proper dimensions.

This description of the eye is in harmony with the recent theory as to the origin of the compound eye offered by Watase ('90), to whom I am indebted for many suggestions and from whose paper I obtained the idea of the method of representation used in Fig. 85.

In some regards the ommatidea here seem to resemble those of Serolis as figured by Watase ('90), and appear to form one of the simplest compound eyes yet described among the tracheate Arthropoda.

No trace of any post-antennal organ occurs.

Genital Organs.

The position of these organs and their histological structure is nearly the same as in Anurida, and because of this I will here only reproduce two figures given by Grassi ('87 *a*), showing their position and form in the adult male (Fig. 86) and young female (Fig. 87). These figures do not represent those portions of the ducts which are of epidermic origin.

General Considerations.

Lepisma is evidently more highly organized in many respects than Anurida. The presence of an ovipositor, of scales, of well-developed mouth-parts, of Malpighian tubules, of a posterior muscular portion of the heart, of a tracheal system, the modifications of the different portions of the digestive tract and the histology of the brain, all attest the considerable differentiation of Lepisma. That Anurida is also quite highly organized is shown by the reduction in the number of abdominal segments and the corresponding condensation of the nerve ganglia, by the presence of various well developed sense organs, and by the presence in the embryo of a "spring." That Anurida is a degraded form is shown by the absence of this spring in the adult, by the great simplicity of the mouth-parts and entire alimentary canal, and by the total absence of tracheæ. These modifications, however, are only such as might be expected with a change of habits and food, and indicate that an original

increase in complexity was followed, after a time, by retrograde development.

Existing Views as to the Relationships of Arthropods.

This portion of my subject has been prepared from material collected from so many sources that it is impossible to give the exact references for each statement. A list of the literature examined, however, will be found at the end of the article.

The division of the Arthropods into Crustacea and Tracheata is one that has long existed.

The latter consists of three large groups—Hexapoda, Arachnida and Myriapoda—and a number of smaller ones, and the various views expressed as to the relations of these subdivisions have differed with the opinions of their authors as to how far the Tracheata might be accepted as a natural group, the general opinion being that the group was by no means to be accepted as such.

Hæckel ('66) advanced the view that all the Tracheata originated from a common ancestor—some primitive crustacean which he termed Zoëpoda, a view already suggested by Fritz Müller ('64). He regarded the Myriapoda as closely related to the Hexapoda; while the Arachnids, though derived from the same stem, diverged from that stem very early in its development. This is the so-called Zoëa Theory.

More recent studies have resulted in the promulgation of another view—that the Insects and Myriapods had a common but far remote ancestor, derived from some primitive Arthropod, from which were also developed the two branches of Crustacea and Arachnida and which was itself derived from some primitive segmented worm.

Before considering these and other views more fully, let us ascertain the prevailing opinions as to the relations of the smaller groups of Arthropods to the larger main trunks.

Limulus is no longer a form of doubtful affinities, the works of Lankester, Kingsley and others having pretty conclusively demonstrated its close relation to the Arachnids and especially to the Scorpions. Near it belong the now extinct groups of Trilobita and Eurypterida. The former is regarded as a branch of the stem from which the Eurypterida developed as a later branch and of which Limulus represents the tip.

The Pygnogonida were at first regarded as Crustacea and later as Arachnida. The researches of Dohrn ('81) have led him to the belief that they are entirely independent of all the other groups of Arthropods. Schimkewitsch ('84, '87), on the other hand, regards them as both primitive and degraded forms, but having common ancestors with the Arachnids, and Morgan ('90) adopts nearly the same view.

The Tardigrada are placed among the Arachnida by Claus ('85), but Oudemans ('85) believes that they should be removed altogether from the Arthropods and placed among the fresh-water Chætopod worms. Weissenborn ('87), from a study of their dermal skeleton and appendages, is inclined to believe that they are offshoots of a branch of the primitive Articulate stem, given off before the Arachnid branch and perhaps before the Arthropod branch. On the whole, the phylogenetic position of this group may be considered uncertain.

Of the groups of the Arachnids—the Araneida, Acarina, Phalangida, Pedipalpi, Scorpiones, Pseudo-scorpiones, Solpugidæ and Pentastomida—the position of the latter group is doubtful, showing, as it does, many features similar to those of the Myzostomida (Balfour, '80); Schimkewitsch ('87), however, regards the proposal to remove them from the Arachnids and place them with this group as unwarranted.

As to the other groups mentioned, the general opinion is that all are derived from a common Arachnid ancestor. Oudemans ('85) dissents from this, however, regarding the Araneida and Acarina as independent groups with no common ancestor nearer than the primitive Arthropod stem.

Peripatus is in many features worm-like; in others, Myriapod affinities are suggested, while still others appear to point to an independent origin. It is generally placed near the Myriapods and Insects, however, and is considered to be a very primitive form.

Passing now to the larger groups, the phylogeny of the Insects is a problem presenting much difficulty in its solution. That portion of the view of Hæckel ('66) which concerns the Insects —that an aquatic Zoëa-like form assumed a terrestrial habit and acquired a tracheal respiration—is, for reasons which will presently appear, unsatisfactory.

Another theory—the "Campodea Theory"—has been advanced by Brauer ('69), Lubbock and Packard. This assumes that the quite general presence, in widely separated groups, of an active, hexapod, air-breathing larva (campodea-form or leptiform larva) is an indication that the earliest insects had similar characters. The caterpillar and grub (eruciform larvæ) are secondary adaptations, according to this view, and the Thysanura are the most accurate representatives of the ancestral form of any insects now living.

The derivation of this form is not agreed upon by the supporters of the Campodea theory. Meyer ('76) believes it to have arisen from some unjointed worm which formed a point of divergence for all Tracheates and the higher worms, and closely related to a form which was the ancestor of the Crustacea. Brauer ('85) regards the Insects as derived from a form in which development of the appendages of the first three post-cephalic segments and retrogression of those of the other segments occurred. From this modified form, through the multiplication of homonomous segments, the Myriapods branched off on one side and the Insecta Apterygogenea (primitively wingless insects) on the other. Lubbock ('74) is inclined to trace the Thysanura back to the Tardigrada, and through them to the Rotifera, while Wood-Mason and Grassi (Haase, '86) derive them from the crustacean Nauplius or Zoëa. Several slight modifications of these views are held by various students of the subject.

A third theory has also been advanced, chiefly by Gegenbaur ('70). He regards the ancestors of Insects as aquatic, breathing by a row of metamerically placed tracheal gills on each side of the body, as is seen in Ephemerid larvæ at the present day. This form developed from a worm bearing dorsal gills. As this worm for some reason became terrestrial, the pairs on the mesothorax and metathorax became greatly enlarged and functional for locomotion, while the others became torn off, leaving an opening where the tracheal trunk passed from each gill into the body, as the representative of the stigmatal opening.

Something not unlike this is met with in the writings of Oken ('47), where he says: "The wings of insects are branchial laminæ converted into air-organs," and Owen ('55) carries the idea a little farther when he says that the wings are "the

homologues of the tergal branchiæ of the vermiform Articulata, raised to a higher function in correlation with a generally transmuted state of the rest of the organization, which is advanced to the utmost perfection of which the Arthropod type of structure is susceptible." The same general conclusion is also reached by Plateau ('71).

This view is a most pleasing one, offering, as it does, such a simple explanation of the origin of wings. The evidence, however, appears to oppose it. If true, the Ephemeridæ would be isolated forms which have retained very primitive features. Yet this group is in many regards quite highly organized. This view also requires that the Insect ancestor should be aquatic, while the earliest fossil remains are those of terrestrial forms, resembling the Orthoptera (Blattidæ). Then, the value of tracheal gills as an ancestral feature is overestimated, for, in some species, precisely similar gills occur on the joints of the legs, on the ventral surface of the body, and even between the wings. As these cannot correspond to the dorsal gills of worms, and as tracheal gills of any kind occur in so few forms, the suspicion arises that these gills, whatever their position, are secondary adaptations, correlated with some change of environment or habits (Graber, '77).

Examining, now, the various views as to the relations of the Myriapods to the other Arthropoda, we find a great hesitancy on the part of the more recent writers to express their opinions.

Placed by all the earlier students of the group as an offshoot of the hexapod stem, or even as the direct ancestors of hexapods, views opposed to these have been very cautiously expressed and then qualified by statements that too little is known of anatomy and development to warrant a discussion of the question. Heathcote ('88) states, however, that he regards them as being "remotely descended from some Peripatus-like stalk," but says nothing as to their relations with the Hexapoda. Packard ('83) believes that the Myriapods branched off from a much more primitive form than the Scolopendrella-like ancestor of the Hexapoda, while Kingsley ('85) voices a growing idea among recent workers when he says, "it seems possible that they (the Myriapods) have no connection with the Hexapods." Peripatus seems to be nearer to the Myriapods than to any other group, and it may be

that these are derived from a common ancestor wholly distinct from that of the Hexapods.

A consideration of the relations of the various groups is best accomplished by an examination of their stem forms. When these are not in existence their characters may be ascertained in part in three ways—from paleontology, and the anatomy and embryology of their descendants. In the examination of these sources of evidence all secondary and recent acquirements, all falsifications of the record, precocious developments, cœnogenetic and all other modifications must be considered. Difficult as the task is, it is rendered far more so by the terrestrial habitat of the Tracheata, for geological evidence points to far more frequent and extreme changes in a terrestrial than in a marine environment, these changes having of course much significance in the struggle for existence and the survival of the fittest. It is not to be wondered at, therefore, that the various views offered in the past have differed greatly in many regards, and any suggestions offered, with our present knowledge, should be only tentative.

The evidence afforded by paleontology is, as for other groups, very fragmentary. Scudder ('86) says: "The earliest insects, then, were generalized hexapods, Paleodictyoptera, in which the four wings were equally and simply developed, membranous, and with very simple neuration. Their metamorphoses were simple and 'incomplete,' the young leaving the egg in the form of the parent, but without wings, the assumption of which required no quiescent stage before maturity. They appeared probably as early as any land plants, certainly by the middle of the Silurian epoch, and continued as a homogeneous type until the end of the Paleozoic period." In Mesozoic times active differentiation occurred, the modern types of Orthoptera, Neuroptera, Hemiptera, and Coleoptera appearing, while the Diptera, Hymenoptera and Lepidoptera appeared last in the Lias and Oölite. Since the Tertiary epoch little change in the general features of the insect life has occurred.

Brauer ('86) regards this view of Scudder's as incorrect. Instead, he thinks that the paleontological evidence shows that Paleozoic insects do not contradict the ordinary belief of a Thysanuran-like ancestor, nor do they form a special order, the common basis of those now existing. He also claims that no proof can be advanced for the group of Paleodictyoptera.

Thus the chief facts derived from this source of evidence are that the oldest known insects were terrestrial, with four similar wings, incomplete metamorphosis, and that they were already quite highly developed in very early times.

Thus far paleontology aids us. Let us now consider the evidence afforded by anatomy and embryology.

Features universal among insects which have many widely differing modifications in connection with differences of food, habits, environment and the like may be considered as probably of phylogenetic value. Hence, to determine the characters of the ancestors of the Hexapoda, these universal features must be collected.

Excluding the Thysanura from consideration, we find the following characters present in all adult insects:

1. The body is segmented and bilaterally symmetrical. Division into head, thorax and abdomen is also an almost universal feature. A pair of jointed legs is present on each thoracic segment. Abdominal legs are absent in the adult. In the embryo, however, a pair of processes appears on the ventral surface of each abdominal segment. These afterwards disappear, and some of the secondary sexual appendages of the abdomen are regarded by some as being derived from them. The presence of wings or of dorsal appendages on the two posterior thoracic segments is very general. On the head occur a pair of jointed antennæ and a pair of eyes. The mouth-parts differ much in the different orders, but may all be reduced to modifications of a mandibulate type, with three pairs of jaws; and that this condition is the more ancestral is supported by what paleontological evidence we possess.

Exceptions to some of these statements occur, but in every case the reason for the exception can be shown to be secondary adaptation, which does not invalidate the statements above.

2. The external covering consists of a chitinous layer secreted by a subjacent cellular layer. Scales and hairs (except sensory hairs ?) are not ancestral.

3. The digestive system varies much in the different groups, the variations being generally correlated with differences of food and habits. Thus in the suctorial mouthed orders there is either a separate chamber, the sucking stomach, with a duct

from it to the main alimentary tract, or the corresponding region of this main tract becomes so modified as to form a series of chambers serving a similar purpose. Those insects with comparatively innutritious food have long and convoluted alimentary tracts with various valvular structures to aid in extracting nourishment from the food, while insects in which this is quite concentrated have simpler digestive organs.

The universal features of the alimentary canal are partially indicated by embryology. From a study of its development we conclude that a median portion is derived from the hypoblast (mesenteron), and that this portion establishes communication with the exterior by means of two invaginations of the outer surface of the embryo, one anterior (stomodæum) and one posterior (proctodæum). These push inward and unite with the mesenteron, all together forming a continuous tube. The lining walls of the two invaginations preserve their ectodermal property of secreting a chitinous outer layer, which is evident in the adult. By modifications of these three parts of the alimentary canal the many differing structures seen in different insects are obtained. Quite universal is the presence of a pair of glands opening into the anterior (stomodæal) invagination, and of one or two pairs of long thread-like tubes opening into the posterior (proctodæal) invagination. While these may possibly be modifications of another set of structures, they apparently assumed their present functions and position at a very early period of insect phylogeny and may fairly be regarded as quite ancestral.

4. The body cavity is at least partly filled by a fat body, in the cavities of which a circulation of blood occurs.

5. The respiration is tracheal, the only exceptions being in degenerate or otherwise secondarily adapted forms. Whether tracheæ leading to open stigmata or into closed gills is the ancestral condition, is a much controverted point which will be considered later. That a tracheal system of some sort is an ancestral feature, however, there can be no doubt, nor that these tracheæ were present in pairs in the thorax and in the anterior (perhaps all) segments of the abdomen.

6. The circulatory system in insects varies little. It consists of a dorsal vessel or heart which becomes contracted in the thoracic region, forming a smaller tube, the aorta. This was probably very nearly its ancestral form.

7. The nervous system of the Hexapods has certain features common to all groups. These are a ganglionic mass above or anterior to the œsophagus—supraœsophageal ganglion or brain—connected with a similar ganglionic mass posterior to the œsophagus—infra or subœsophageal ganglion—by two nerve cords, one passing each side of the œsophagus, which is thus encircled by a ring of nervous matter. The two nerve cords constitute the circumœsophageal commissures. Posterior to the subœsophageal ganglion is a series of similar ganglia joined to each other by two commissures. Comparison of the nervous system in different insects and in different stages of development indicates that the ganglia, while showing a tendency towards a metameric arrangement, one for each segment of the body, show also a tendency to concentrate towards the head and fuse with one another. Resulting from this, the number of ventral ganglia varies in different insects. Similarly, some insects have ganglia which are dense rounded masses of nerve cells and fibers; in others these show traces of having been at some time two ganglia, side by side; and in still others the fusion is even less complete. Thus we may assume as primary two parallel bands of nerve cords, with an enlargement in each band anterior and another posterior to the œsophagus, and one enlargement in each of the remaining segments of the body. Probably the two ganglia anterior to the œsophagus had already fused to form the superœsophageal ganglion. The subœsophageal ganglion is probably the result of the fusion of the ganglia of several segments, but this condensation was doubtless accomplished at a very early period.

8. Insects are always unisexual. The essential organs are paired or show traces of modification from such a condition, and open to the exterior by median ducts of epiblastic origin (certain exceptions do not affect this general statement). The secondary sexual features differ greatly in different insects and have no phylogenetic value, being comparatively recent acquisitions.

Some of these points demand a fuller consideration. The question as to the significance and homologies of the parts of the head and mouth is one of much importance, for, if it can be shown that the Arthropod head consists of a series of modified and much condensed metameres only, it will aid in establishing certain views as to the phylogeny of the group, to be considered later.

Everywhere among the Arthropods we observe metamerism which, in the thoracic and abdominal regions, is almost wholly undisguised. We find that these regions consist of a series of similar segments, showing much the same structure, both as regards the skeleton and the internal anatomy. Each segment bears a pair of ventral appendages; each has a nerve ganglion, and the appendages of a segment are innervated from the ganglion of that segment. In the insects the thoracic appendages have become greatly developed; with the abdominal appendages, on the contrary, it is the reverse, and their existence is now shown chiefly by embryology. A study of the mouth-parts shows that they are ventral appendages which have been modified from an original locomotor function, and a study of their number and innervation should throw some light upon the true value to assign to the head, indicating the amount of cephalization and condensation it has undergone, and of what it really consists.

In side view the supraœsophageal ganglion of insects shows three enlargements. The anterior one, called by Viallanes ('87) the protocerebron, gives rise to the nerves which innervate the eyes; the middle enlargement—deutocerebron—innervates the antennæ, while the third—tritocerebron—which in the Decapod Crustacea innervates the second pair of antennæ, here innervates no appendages, but gives rise to the roots of the stomato-gastric (sympathetic) system. On this segment is borne the labrum or upper lip. This is not regarded as a pair of fused appendages, however, but as a modified portion of the ventral integument. Passing to the subœsophageal ganglion we find that it innervates the mandibles, maxillæ and labium, indicating that it is the product of a fusion of three distinct ganglia. If this be correct, the view of Viallanes must be accepted and the head be considered as being composed of six segments, three preoral and three postoral.

The origin and homologies of the respiratory system are much disputed points. The view of Gegenbaur ('70), already briefly stated, is that the open system with stigmata is secondary, the primary condition being a closed system leading into a pair of lateral leaf-like expansions on the side of each segment. The air in the tracheæ in these expansions was thus brought very

close to that in the water in which these insects lived, and by a gaseous exchange that within the tracheæ was purified. He believes that the tracheæ originally functioned as hydrostatic organs and became secondarily adapted to their respiratory function, developing for its furtherance the lateral outgrowths. Of these, two pairs on two thoracic segments became hypertrophied upon a change to terrestrial life, while the remaining ones became lost at a subsequent molt of the cuticle, leaving open stigmata.

The objections to this view are several. One of Gegenbaur's arguments to support it is that larvæ which will later have appendages of the kind under consideration, on certain segments, have no stigmata on these segments. As by the theory the wings are hypertrophied tracheal gills, stigmata should not occur on these segments. Quite a number of beetle larvæ, however, are known which have mesothoracic stigmata. Another difficulty has been brought forward by Palmén ('77), who has shown that, though the gills of some aquatic larvæ are attached to the skin near where the stigmata will open in the adult, and though stigmata and gills never coexist in a segment, yet the point of attachment of the gill never coincides with the place where the future stigma will open. He urges that the gills and stigmata have no genetic relation and that the closed tracheal system is secondary.

The development of Calotermes as given by F. Müller ('75) is also opposed to this view. In the young larvæ wing-like thoracic processes appear, into which the tracheæ grow at a distinctly later period. If Gegenbaur's theory were correct the lateral processes should contain tracheæ from the first, as the processes, according to that view, are developed to satisfy the demand for the purification of the air in the tracheæ. From his studies on the subject Müller thinks that the wings are not modified tracheal gills, but regards them as derived from lateral processes of the dorsal plates of the segments concerned.

The evidence from rectal gills in the larvæ of the Libellulidæ points to the closed system with a rectal place of purification as being secondary and correlated with the assumption of an aquatic habit. If this be so, it is equally plausible that metameric external gills are also secondary acquisitions, and other facts

seem to sustain this view. Hence the assumption above, that the primitive form of respiration was through open stigmata, and that the forms possessing it were terrestrial, is not disproved by the facts.

The absence of stigmata on the head and on one or two abdominal segments remains to be considered in this connection. The view which regards the Insect as a series of similar metameres throughout, demands that each organ, segmentally repeated in some segments, should be present in some form in every segment. To find traces of the missing stigmata and the tracheæ leading from them, embryology has been resorted to, and in some cases with success. For the lack of stigmata on certain abdominal segments, the description of the development of the Bee, as given by Grassi ('86), may be considered. He says that on the posterior region of the dorsal surface of the embryo, two little pits are formed. Later, the area containing them becomes invaginated to form the proctodæum, and elongation of the pits converts them into the two pairs of Malpighian tubes of the adult. The only difference between these pits and those of just developing tracheæ is that the former are nearer the middle line of the body, but if the abdomen were not folded onto the dorsal surface of the egg the pits would exactly correspond. He claims additional weight for this explanation from the fact that in the silk-worm stigmata are absent from these segments and there are three pairs of Malpighian tubes in the adult. On the other hand, Cholodkovsky ('87) states that the larval Malpighian tubes of each side, normally three in number, join the proctodæum by a single basal trunk. In the pupa the terminal portions undergo histiolysis and are lost, while the basal trunks grow out to form the Malpighian tubes of the adult, remaining simple (Tineidæ) or branching to form three on each side (most Lepidoptera). If these observations be correct the primitive condition in Lepidoptera was that of a single pair of Malpighian tubes, and therefore representing the stigmata and tracheæ of but one segment.

If we seek for modified respiratory organs in the head we find no structure which can be homologised with them in any part of the preoral portion. Homologous structures are apparently present in some of the postoral segments, however.

Two organs may be considered in this connection—the salivary and the spinning glands. The former arise as paired epiblastic invaginations of the ventral plate, just behind the mouth, on the inner side of the mandibles. The ducts afterwards unite, forming a common duct which acquires an opening into the mouth. They are lined by chitin which shows markings very similar to the delicate spiral threads so characteristic of tracheæ. The generally accepted opinion is that the salivary ducts (and probably the glands) represent the tracheæ of the mandibular segment of the head.

The consideration of the spinning glands is more difficult. They are present, according to Claus ('85), only in larvæ, but more recent researches have established their presence in the adult Tenthridinidæ (Poletajew, '85), and in embryonic life Grassi ('86) has described in the Bee three pairs of epiblastic pits corresponding to the buccal segments. That one well developed pair of spinning glands occur, as already stated, in larvæ, is well known. These arise as epiblastic invaginations on the inner sides of the second maxillæ (labium) in Apis and Lepidoptera, according to Balfour ('80), and "are very similar in their structure and development to salivary glands."

These facts indicate that spinning glands, while of great use to some larvæ, have become rudimentary and only perceptible in the embryo in the majority of insects. The three pairs of pits observed by Grassi would indicate the presence of a maxillary pair of tracheæ which have become even more rudimentary than the labial pair. It would be interesting, however, to study the development of the salivary reservoirs, to determine whether they are diverticula of the salivary ducts or derived from maxillary invaginations which have acquired a secondary relation to the mandibular pair.

Thus we find at some stage of development traces of structures homologous with stigmata and tracheæ in each of the postoral segments of the head. Their absence on the preoral portion, and the absence there also of ventral appendages innervated by the brain, indicate that, while it is possible that the preoral portion is itself a fusion of several (three) segments, it is also possible that these segments are not homologous with the postoral ones. What may perhaps be interpreted in the same way is the

absence of nephridia from the three anterior somites of the body of the Oligochæta.

The primitive arrangement of the genital ducts remains for consideration. The almost universal unpaired condition finds an exception among the Ephemeridæ, where these ducts are distinctly paired, as has been shown by Palmén ('84). Considerable embryological evidence also indicates a paired arrangement as being the primitive one. Without going into details on this topic, suffice it to say that it is probable that the genital organs and ducts were primarily distinct from each other throughout.

If from this summary of the ancestral features retained by the insects now existing we attempt to describe the structure of the primitive form from which they have been derived and which has been termed Archentomon by Meyer ('76), we shall obtain the following:

Archentomon was a segmented bilaterally symmetrical animal, showing a division into head, thorax and abdomen (perhaps slight). A pair of jointed legs occurred on each thoracic and abdominal (?) segment; two pairs of wings (perhaps only dorsal lobes) were present on the last two thoracic segments; a pair of antennæ and a pair of eyes were borne on the head; the mouth-parts were formed for biting, and three pairs were present. The body was covered by a somewhat chitinous layer resting upon a cellular layer; the alimentary tract was a straight tube, the terminal portions of which were lined by a chitinous layer like that of the surface of the body. Into the anterior portion of this tube opened the ducts of a pair of secretory glands, and into the posterior portion opened several thread-like hollow tubes. Respiration was effected by tracheal tubes, opening to the exterior, one pair in each thoracic and abdominal segment except one or two of the latter.

The circulatory system consisted of a dorsal vessel, narrowed in the thoracic region. The nervous system consisted of two fused anterior ganglia, forming a supracœsophageal ganglion; two nerve cords, one passing each side of the œsophagus and entering a subœsophageal ganglion consisting of several ganglia fused; posterior to this a segmentally placed series of ventral ganglia joined to those next in front and behind by pairs of commissural bands. A fat body at least partly occupied the body cavity.

The sexual organs were paired, opening to the exterior by a median duct. The sexes were distinct and the animal was terrestrial.

Having now obtained some idea of the probable characters of the earliest insects, we are prepared to consider the relations of the Thysanura to these forms.

If we inquire into the cause of simplicity in some members of a group of in general high complexity, we find that this simplicity may result from the action separately or together of a number of influences. It may be due to a simple retention of ancestral features, the animals concerned entering but slightly into the struggle for existence with other species, or living under circumstances which have changed but little (as in Limulus), or it may be the result of degradation. If the latter be the case, the degradation may be the result of a parasitic life, or of a change from a motile to a fixed life as in the barnacles (Cirripedia), or it may be correlated with a change of habits and surroundings. Restating these possibilities for the case in hand, and excluding degradation resulting from parasitism and from an attached life, as not here applicable, we obtain the following. The Cinura and the Insects are derived from a common stock. *a*. Both groups have undergone much differentiation, but in the Thysanura this ceased after a time and was followed by degradation, resulting in the production of forms so simple as to appear primitive. *b*. While the Insects have undergone much differentiation, the Thysanura have from some influence varied little and have retained most of their ancestral features. If the former view concerning the Thysanura be correct, a study of the embryology of these forms should show us traces of a greater differentiation, followed by modification and retrogression. It is true that little is known of Thysanuran embryology, but that little shows us no such changes as this, but instead a constantly increasing differentiation until the embryo leaves the egg, when it is nearly like the adult. Much of the same reasoning that regards Limulus as having remained in so nearly its primitive state because of the uniformity of its environment, will apply with nearly equal force to the Cinura, and the conclusion we reach is that the group has retained most of its ancestral features and is composed of really very primitive forms.

With the Collembola the case is somewhat different. To many features common to both groups are added others suggestive of a greater amount of differentiation. Thus we find a concentration of the abdominal somites, and, correspondingly, of the nerve cord; the presence of various well developed sense organs, and of a complex structure—the spring.

In some cases some amount of retrogression is also noticeable, as in Anurida. The absence of a spring in the adult, of tracheæ, and the simple condition of the mouth-parts, all indicate degradation. The general belief, drawn from a consideration of these facts, is that the Collembola is a group in which greater differentiation has occurred than in the Cinura, followed, however, in some cases by a degradation.

We may therefore conclude that in a general way the Campodea theory is sustained on the one hand by the evidence obtained from the higher insects, and on the other from the anatomy of the Thysanura. Of the latter group the Cinura are less complicated on the whole than the Collembola, and Campodea and Japyx are among its simpler members, while Lepisma and Machilis are more highly developed.

An attempt to construct a genealogical tree in accordance with the views thus far accepted would result about as follows: From Archentomon two or several trunks developed. One of these trunks divides, one limb becoming the Cinura, which have become only slightly modified from their Archentomon ancestor. Hence the branches of this limb would be short, the longest ones representing Lepisma, Machilis, etc., while the tip of the limb itself would probably represent Campodea or possibly Scolopendrella, which will be considered below. The other limb would represent the Collembola and send branches in various directions. Some of these branches, however, soon bend back towards the limb, while all diverge but little after the first. The other main trunk or trunks develop rapidly and in different directions, and represent the different groups of the higher insects. Whether they were derived from one trunk or several cannot here be discussed.

For years the Campodea theory rested at this point. Attempts to trace relationships with Crustacea, Tardigrada, and other groups were made, but no results of value were obtained.

Ryder ('80), however, upon examination of Scolopendrella, a little animal described by Newport ('44) as one of the Chilopoda, came to the conclusion that it should be regarded as the type of a new order of Articulata, for which he proposed the name Symphyla. This form has come to be regarded by many as the probable survivor of the group of animals from which Archentomon was descended, while in our systematic works it is included among the Thysanura as a suborder equivalent to the Cinura and Collembola. Ryder ('80) says: "it may indeed be looked upon, perhaps, as representing the last survival of the form from which insects may be supposed to have descended."

This view is at least sustained in part by the anatomy of Scolopendrella. It consists of a head and thirteen body segments, each bearing a pair of legs; the head is flattened; the antennal insertion and shape is as in Campodea; the mouth-parts consist of a labrum, mandibles, maxillæ and labium; the legs each end in a pair of claws, as in insects; the genital opening is indeed ventral, but is situated in the anterior portion of the fourth segment; a pair of stigmata situated on the sides of the head lead into tracheæ which pass back to the fourth body segment; the intestine is straight and has two Malpighian tubules. These and other anatomical resemblances to the Thysanura have led to a quite general acceptance of Ryder's opinion, as above quoted, despite the lack of division into head and thorax, the presence of functional legs on the posterior segments, the abnormal location of the genital opening, and other characters suggestive of Myriapod affinities. These features have been considered as indicating a close relation between the Myriapods and Hexapods.

Let us now consider the Myriapods with reference to a possible relationship with the Insects. Here we find the head distinct from the body, but no division of the latter into thorax and abdomen. In this regard Scolopendrella approaches the Myriapods rather than the Hexapods. In the internal anatomy of the Diplopoda (Chilognatha) the fusion of some of the anterior ventral nerve ganglia may perhaps be an indication of thoracic and abdominal division, but external anatomy fails to confirm this view.

Perhaps the most noticeable distinction between the Myriapods

and Hexapods is that expressed by the names of the groups. Those persons who have attempted to derive the latter from the former have laid stress upon the presence of abdominal appendages in the embryo Hexapod, and especially in mature Thysanura, where they are regarded as having been retained for some reason in the adult, though absent in this stage in the Insects. The presence of two pairs of feet on most of the segments of the Diplopoda has also been called attention to as indicating a vegetative increase in the number of segments and thus explaining the divergence in the number of segments of the two groups. This view as to the original condition of the segments has been upheld by Balfour ('80) and Packard, while Newport regards the double segment as the consequence of a partial fusion of two which were originally distinct. Recent studies by Heathcote ('88) on Julus show that the nerve cord, tracheal and circulatory systems and the segmentation of the mesoblast indicate the correctness of the opinion expressed by Newport: the only simple portions of the segments are the dorsal plate and the stink glands. Heathcote considers each part as representing a complete segment, but joined to its fellow by the fusion of the dorsal plates.

Comparative studies on the Myriapods appear to indicate that the Diplopoda are the more primitive, but with a number of secondary modifications, and that the Chilopoda are a quite divergent group. If the assumption of a vegetative multiplication of segments be correct, we must also assume that in the Diplopoda it has been followed by a change in the opposite direction, a tendency towards a reduction in the number of these segments. Considering what value should be given to the presence of abdominal appendages in Hexapods, we find that in insects the appendages appear as small buds which remain incomplete and have but a brief existence. These facts are discussed by Graber ('88), who regards them as showing that the Insects are derived from ancestors with well developed abdominal appendages serving a definite function. What this function may have been is uncertain. Naturally the first thought would be that they were true legs, but the form of the buds of the first abdominal segment is sack-like or leaf-like and gives not the slightest indication of a locomotor function. This, however, Graber considers as being

possibly the result of a secondary modification of this pair of appendages. If not, the view that they were legs must be abandoned and some other function substituted, such as branchial respiration, in which case the appendages might be interpreted as gills.

Examination of the appendages in the Thysanura is next in order. This has recently been accomplished by Haase ('89), who finds small abdominal processes in the Cinura, Collembola, Symphyla and in a few Insects. The conclusions he arrives at are that the Thysanura or their ancestors possessed abdominal appendages; that these became rudimentary and in many cases entirely disappeared. The present existing abdominal appendages of the Thysanura he regards as not homologous with those formerly possessed. In his own words: "Aus diesen Ansichten über die allmähliche Reduktion der Abdominalbeine erhellt auch, dass die beweglichen Griffel nicht als Rudimente von Beinen, sondern nur als diesen Analoge, aber nicht einmal homostiche Neubildungen anzusehen sind, die wohl auf besondere Entwicklung paariger Hinderrandsborsten auf den noch deutlich vorspringenden Extremitätenresten zurückzuführen wären." Supporting this is a statement by Oudemans ('89), of which the details are as yet unpublished, that Thermophila funorum, one of the Lepismidæ, which, when adult, has a pair of "zapfenartig" appendages on the ventral sides of the seventh, eighth and ninth abdominal segments, is without these in immature specimens. The hinder pair appear first, then those of the eighth segment, and finally the anterior pair. This is equally true of both sexes. Oudemans claims that if these were the rudiments of true legs, as is usually claimed, they should appear more nearly at the same time and in the reverse order, and therefore concludes that they are not rudimentary and that they have nothing to do with true abdominal legs. Another consideration is that if homologous with the abdominal appendages of insect embryos, these last appear at such an extremely early period of development that we should expect to find those of Thysanura appearing before the adult condition is reached. In other words, the tendency of a character to appear earlier and earlier in ontogeny seems insufficient to account for the great distance between the embryonic stage at which these buds appear in Insects and the adult condi-

tion as seen in forms differing in no greater degree than do Thysanura and Insects.

Another feature worthy of consideration is the embryology of the Myriapoda. The memoirs of Newport ('41) and Metschnikoff show us that in this group the larva emerges from the egg with less than ten post-cephalic segments and with three pairs of well developed legs. Additional segments and appendages develop as post-embryonic changes. If we compare this process with that in Insects we find a radical difference. In the Insect embryo, before hatching, rudiments of appendages appear to the number of ten pairs upon the abdomen (Lepidoptera) aside from the usual thoracic pairs. After hatching, instead of an increase in the number of segments, either no change occurs or there is a reduction, never an increase, while the abdominal appendages become lost before the hatching takes place.

Another difficulty is met with in attempting to compare the appendages of the head and thorax in the two groups, and on this subject I quote Moseley ('84), who says: "The young Strongylosoma has three pairs of functionally active legs; the young Julus has a similar number, the third post-cephalic somite being apodous, and it was formerly thought this fact established a connection between the Myriapoda and the Insects, the three pairs of larval legs of the Myriapod being supposed to correspond with the three pairs of legs of Insects. Such, however, is not the case. There being no second maxillæ in Myriapoda, the first pair of legs in the larva must correspond with the second maxillæ of Insects; and even if this could be shown to be incorrect, the three pairs of legs would still not correspond with those of Insects, because in the young Strongylosoma the second, and in Julus the third, post-cephalic somites are devoid of legs. Moreover, the larvæ are only apparently hexapodous, not in reality so. In Panropus there is a hexapodous larval stage; in the Chilopoda no functional or real hexapodous larval stage occurs, as far as is known."

The mechanism of respiration in the Myriapoda has been investigated by Charlande ('87) and found to differ entirely from that in the Hexapoda, for in the former group no dilatations or contractions of the body cavity capable of causing respiration occur during repose: neither the stigmata nor the sub-stigmatal

membrane move in any relation to respiration, the stigmata being wholly passive. In repose inspiration and expiration are caused wholly by rhythmical movements of the dorsal vessel: in motion this is aided by the action of the muscles on the tracheæ, and during digestion by movements of the digestive tract also. In the Myriapods the antennæ are innervated from the brain anterior to the portion innervating the eyes, according to Newport ('43), but in the Hexapods, according to Kingsley ('85), a different arrangement apparently holds. My own observations fail to confirm this last statement for the Thysanura at least, and the subject needs re-examination.

These differences are so great, both in number and amount, that a close relationship of the Myriapoda and Hexapoda appears doubtful, and the position of Scolopendrella becomes more uncertain. We may conclude that the weight of evidence negatives any close relationship at least, and our knowledge is too insufficient to determine whether there is any relationship whatever.

Turning now to Peripatus we find, as in the Myriapods, no division into thorax and abdomen, and that the body is a repetition of similar metameres, each with a pair of appendages. The head bears a pair of jointed antennæ; the mouth is armed with a pair of jaws, and is followed by a pair of papillæ, on which open the ducts of a pair of slime glands.

Without attempting a description of the anatomy of this interesting animal some features may be touched upon. In the Insects we have three pairs of mouth-parts, in the Chilopoda three, in the Diplopoda two, and in Peripatus a single pair. The number of pairs of antennæ is the same throughout. The nervous system of Peripatus is unlike that of any other known Arthropod. The supra-œsophageal ganglion is formed by the fusion of two masses, as in the other Arthropods; but the two halves of the ventral nerve cord, instead of being fused and situated on the median line, lie quite far from each other, and are connected by numerous cross-commissures, giving the entire structure a ladder-like appearance. At the posterior end the two cords bend dorsally and unite above the rectum. The sexual organs open to the exterior near the posterior end of the animal by a median duct. In the Diplopods the sexual organs form a single median mass opening to the exterior upon the coxal

joints of the second pair of legs by a pair of ducts. In the Chilopods the organs are unpaired, and open by a single duct at the posterior end of the body. The arrangement in Insects has already been described and its primitive condition considered.

In their development the species of Peripatus are very anomalous and differ greatly. In three species Sclater ('88) finds no yolk and a total segmentation: in P. capensis there are indications of a recent loss of yolk and the segmentation is total or meroblastic, while in P. novæ-zealandiæ there is a great deal of yolk and segmentation is meroblastic. In P. capensis gastrulation is shown by Sedgwick to be epibolic. The elongation of the blastopore and its division result in one part becoming the mouth and the other the anus of the adult. These and other peculiarities make the task of locating of this form a difficult one. One feature of the tracheate Arthropods is the possession of Malpighian tubules. In Peripatus these are wholly lacking, but their function is accomplished by segmental organs comparable to those of Annelids, a pair of which occurs in each of the leg-bearing segments.

In Peripatus we find a pair of slime glands derived from the epiblast opening just behind the mouth. Whether these correspond with the spinning glands of Insects, already considered, is uncertain. They cannot be homologous with the salivary glands, for a pair of these is also present. In Peripatus they lead forward and, as stated, open near the mouth: in Insects this is true of the spinning glands: in Myriapods Tömösvary ('84) has described spinning glands which he regards as epiblastic invaginations, opening at the posterior end of the animal. In Scolopendrella Charlande ('89) has described similar glands opening to the exterior on the extremities of anal appendages, which are considered by Haase ('89) as corresponding to the cerci of Insects.

If these glands are homologous structures in any of these groups their external openings would be expected to be at the same end of the animal. This would class together the Myriapods and Scolopendrella, and Peripatus and the Hexapods—a relationship not sustained by the other facts, at least for the last group. If we remember that in the Spiders spinning glands opening near the posterior extremity also occur, the conclusion appears to be forced upon us that these glands are not homologous and are of little phylogenetic value.

Heathcote ('88), in his studies on the development of Julus, finds points of resemblance to Peripatus, and in conclusion remarks "that the Chilopoda and Diplopoda branched off from some common ancestor not long before the appearance of the Archipolypoda, and that both are remotely descended from some Peripatus-like stock." This view would place Peripatus and the Myriapoda as distantly related forms, to which the Insects have only very distant relations.

It may be well to consider here the structure and homologies of the so-called "ventral sacs," which occur in Thysanura especially. These sacs have been studied by Haase ('89) in various Cinura, in the Diplopoda and in Scolopendrella, together with the structures regarded by him as homologous with them in other groups. His conclusion is that in the groups named they are of the nature of coxal glands, and that the ventral vesicle of the Collembola is of the same nature, with the addition of a secretory function. The sacs have retractor muscles and can thus be drawn into the body. When extended, however, blood may be seen flowing through them, and as the development of these sacs and of tracheæ appears to be correlated, the increase of tracheal respiration coinciding with a reduction of the sacs, he regards these last as blood gills (Blutkiemen). The first pair of abdominal appendages of insect embryos he regards as having also possessed this function at some time, hence their different form from that of the appendages which follow. He also considers as homologous with these the coxal or crural glands of the Diplopoda and Peripatus.

A more generalized view of the subject has been taken by Eisig ('87), who believes that all Arthropods have structures homologous with these sacs. The organs which he regards as homologous are, in Peripatus, the spinning (slime) glands and crural glands; in the Myriapods the spinning glands, part of the crural glands and the ventral sacs; in Scolopendrella the spinning glands and the ventral sacs (which he states are coxal glands); in the Thysanura the ventral sacs; in Insects the spinning glands and the possibly existing coxal glands; and in the Arachnoidea the spinning and coxal glands. If this view be correct the homology of the spinning glands of Insects with cephalic tracheæ is invalidated.

The relation of the groups already considered to the Arachnida must now be discussed. The most marked similarities are the presence of tracheæ or of lung-books, and of Malpighian tubules in all these groups.

The most primitive form of aerial respiration in the Arachnids is by means of lung-books, as is seen in the Scorpions. In the Spiders lung-books and tracheæ may coexist, while in the Mites only tracheæ are present (Schimkewitsch, '84). The origin of the lung-books from involuted appendages, hypothetically assumed by Lankester ('85), has been corroborated by the observations of Bruce ('87), and "the tracheæ of Arachnids are but modifications of the pulmonary organs [lung-books] existing in some of the group." Without discussing this point farther I would refer to the writings of Lankester ('85) and Kingsley ('85) as expressing recent views upon this subject. If then the respiratory organs of Arachnids are derived from the appendages, tracheæ and unmodified appendages should not coexist in a segment. This is the case, however, with Insects, and, as already pointed out by Bruce ('87), the conclusion follows from these facts that the tracheæ of Insects and of Arachnids are probably not homologous, but analogous structures.

As regards the Malpighian tubules they are described in Insects, with but one exception, as being derived from the proctodæal area. Nassonoff (Schimkewitsch '84, footnote), however, states that in ants the tubules of the larva atrophy and the adult ones develop anew, as diverticula of the mesenteron. Contradicting, as it does, the observations of all who have examined the Malpighian tubules in Insects, this view must be considered as needing confirmation, and for the present the tubules may be regarded as proctodæal and therefore epiblastic in origin. In the Arachnids these organs have been studied by Loman ('86–'87), who finds that in every case they empty into the mesenteron just anterior to the proctodæum. If in Insects they empty into the proctodæum, we have here as with the tracheæ a case of analogy rather than of homology.

Another point of difference between the Insects and Arachnids is the lack of antennæ in the latter group, where all the appendages appear to be primitively post-oral, while the antennæ of Insects are primitively pre-oral.

These and other considerations which cannot here be enumerated lead to the belief that the Arachnids have no close relationship with either the Hexapods, Myriapods or Peripatus.

One group of Arthropods remains to be considered—the Crustacea. The old opinion that all Arthropods belonged to a common phylum has of late years wavered before a growing doubt as to the accuracy of this belief. The study of the Hexapods points to a form with many segments as its ancestor, while the Myriapods appear to have developed from a form having a much smaller number of segments than that possessed by the present adult forms. Peripatus, as we have seen, does not agree well with either group in its development nor in some of its adult features. The Crustacea differ from all these groups in a number of regards. Thus the proctodæal excretory tubes of the Hexapods and Myriapods are entirely lacking here, the excretory function being performed in the higher forms by the green glands and in the lower forms by the shell glands. The appendages of the Crustacea are typically biramous, while they are never so (with a few doubtful exceptions) in the other groups. In the Crustacea the proctodæum is usually formed before the stomodæum, while in the other groups the reverse is generally true. Other anatomical and embryological characters also exist which indicate a separation of the Crustacea from all the Tracheata except the Arachnids.

While great differences between the Crustacea and Arachnids also occur, a number of characters point to a closer relationship of these groups than of these and the others. In the Arachnids all the appendages appear to be post-oral in origin, and this holds true of the Crustacea, at least as far forward as to include the second pair of antennæ. Respiration, though branchial in the Crustacea, finds itself paralleled by the Arachnid lung-book, which has already been shown to be an involuted appendage, and this appendage, before its involution, must have had a branchial function. The presence of mesenteric tubules for excretion in the Arachnids corresponds with the presence of similar tubules described by Spencer ('85) as present in the mesenteron of Amphipoda. The shell and green glands of Crustacea have been compared to the coxal glands of Scorpio described by Lankester ('82), and to similar glands in Limulus and the Spiders. Eisig's

view, however, would lead to these glands being looked upon as a general Arthropodan character, and therefore as of less value for the determination of the relations of the smaller groups.

These and the facts previously presented render a discussion of Hæckel's zoea theory unnecessary, and a common ancestor of all these groups must certainly have lived at a period long before the appearance of such a highly differentiated form as the Crustacean zoea.

Perhaps the most recent view as to the relations of the different groups of Arthropods may be expressed as follows: The primitive Arthropod trunk, near its root, divided into two great branches, of which one developed through the Phyllopoda, forming the Crustacea, and early giving off a branch which developed Limulus and the Arachnida. The other great branch, through Myriapod-like forms, developed the Hexapoda.

The weakest portion of this view is its assumption of the development of the Hexapods through Myriapod-like forms, and those who dissent from this view, while accepting the relation of the Crustacea and Arachnida as given above, look upon the Hexapods and Myriapods as independently derived from the same stem as the Crustacea, with Peripatus as also an independent branch of this stem or as a very early offshoot from the Myriapodan branch.

We now confront the question, What is the origin of the Arthropoda? Several attempts, all more or less unsatisfactory, have been made to answer this question.

Assuming the monophyletic origin of the entire sub-kingdom, the most primitive forms of the different classes composing it have been appealed to for aid in the solution of the problem. Embryology has also been called upon to give assistance, for the value of ontogeny as a recapitulation of phylogeny, analogical only though it may be, is universally recognized. In larval stages, where the animal is exposed to the struggle for existence, ancestral features may become modified or concealed by secondary ones. Hence, larval stages which are ancestral can only persist in an unchanged environment. As these changes have been far greater on land than in the ocean, it follows that marine larvæ show more trustworthy features for phylogenetic study than do terrestrial ones, and this explains the importance of determining

the ancestral Crustacean for the light it should throw upon the derivation of the entire group of Arthropoda.

The general presence of a zoea larva in the developmental stages of the Malacostraca for a time led to the belief that this larva represented an ancestral stage in the history of this group. More complete and accurate observations, however, showed that no definitely characterized zoea larva occurred, the forms which had been classed as zoeas being in reality quite different and heterogeneous in different Crustacea. These observations also called attention to the fact that in many cases a simpler "Protozoea" larva was present, appearing before the zoea in ontogeny, and differing from the latter in that it could be clearly distinguished from other larval forms, even in widely differing Crustacea. As a result of these observations the zoea is no longer regarded as ancestral, but has been relegated to the class of secondary larvæ.

The significance of the protozoea is a distinct question. Its slight variability in different forms, however, and other reasons have led to its acceptance as an ancestral form, but not the most ancestral form which ontogeny has preserved, of the phylogenetic development of the Malacostraca. That this is true is shown by the fact that in several widely different forms the larva, which appears on escaping from the egg, is not a protozoea but a simpler form—a true Nauplius, comparable in all respects with the Nauplius larva of the Entomostraca. This Nauplius, having the same characters, whether as a larval Decapod, Cirriped, Phyllopod or Copepod, is generally accepted as representing the most ancestral stage which ontogeny has preserved, and the protozoea as representing a later but also ancestral stage. In most Malacostraca the stages previous to the latter are either omitted, or occur before the escape of the larva from the egg membranes.

The striking resemblance of the protozoea to the adult Phyllopods has resulted in a modification of the view that the different groups of Crustacea diverged from their Nauplius ancestor. Claus ('76) urges that the Malacostraca were derived from some stage subsequent to the Nauplius, which he designates as the Urophyllopod, and regards the protozoea as being the ontogenetic representative of that form. This view would explain the resemblance of the Protozoea and the Phyllopoda, and place

the Nauplius as an ancestral feature common to both branches. The Nauplius is therefore regarded as representing (with a few secondary modifications) the most primitive Crustacean which has been preserved.

In the theoretical portion of his article on the development of Lepidoptera, Hatschek ('77) first raised objections to this view. He claimed that the ventral nerve chain of the Annelids, Crustacea and Tracheata was homologous, and that any phylogenetic view which conflicted with this was untenable. This was the case with the Nauplius theory, for, according to that, the Nauplius represents an ancestral condition of the Crustacea, and as it has no ventral chain this must be a more recent development. But as the Annelids cannot have been derived from the Crustacea since the Nauplius, it follows that the ventral chain in Annelids has been independently acquired from that of the Crustacea. But as the first statement was that the ventral chain is homologous, the Crustacea must be derived from the Annelids, and the Nauplius is secondary. "Zweifellos hatte die Stammform der jetzt lebenden Crustaceen die Naupliusform als ontogenetische Stadium zu durchlaufen."

Since the publication of this article quite a number of biological workers have become inclined to look upon the Nauplius as a secondary, non-ancestral form. One of the most active opponents of the Nauplius theory is Lang, who, in his Textbook of Comparative Anatomy ('89), gives his reasons for the views he holds. These reasons he classifies as general and special. The general grounds for the rejection of the Nauplius theory are: 1. We know of no animal which, in its adult condition, is comparable to a Nauplius. 2. We ought not to draw conclusions as to the early phylogenetic forms of any group of animals without farther knowledge of their early larval stages.

The special grounds he gives are as follows: If the Nauplius theory be accepted, the origin of the typical crustacean organization, of the segmentation of the body, of the segmented ventral nerve-cord, and of the segmented dorsal heart remain unexplained. Then the genital ducts, with a single exception, open on portions of the body which in the Nauplius are undeveloped.

If, on the other hand, we regard the Nauplius as a characteristic larval but not ancestral form for Crustacea, it is easily

explained by the assumption of an annelid-like ancestral form for the group. Just as the ancestral form of the Crustacea leads back to the Annelids, does the larval Crustacean lead back to the larval Annelid. Characters of the adult appear earlier and earlier in development. Thus, in the crustacean larva, which corresponds to the annelid trochophore, crustacean characters have appeared, viz. the three pairs of legs and the dorsal shield. The new mode of locomotion by the legs has rendered the old mode useless, and the ciliated ring of the trochophore has therefore become lost. In the Nauplius the three pairs of appendages become the three most anterior pairs of the adult. Why is it that *these* three pairs and *only* these appear? The answer is that in a young larva escaping from the egg at a very early period, as is the case with the Nauplius, only those organs can have been developed which are essential to an independent life and the obtaining of nourishment. These three pairs of appendages serve for locomotion and also, in part, as aids in capturing and conducting food to the mouth, around which they are situated. When the egg has more food-yolk the larva escapes as a Metanauplius with the foundations (Anlagen) of other appendages.

As a typical crustacean larva the Nauplius shows many original crustacean characters, notwithstanding, as the dorsal shield, the unpaired eyes, the frontal sense organs, the special structure of the hinder antennæ, and mandibles, etc. In conclusion, Lang says: "Der Nauplius ist also auf eine Trochophoralarve zu beziehen, in welche schon Krebscharaktere zurückverlegt sind, sie ist ungegliedert, enthält die Anlagen des vorderen Kopftheiles der erwachsenen Krebse mit dem Munde und die Anlage des hintersten Leibesendes mit dem After. Zwischen beiden liegt eine embryonale Bildungszone von der aus bei der Weiterentwickelung der Larve sich der übrige Körper anlegt und wie bei den Anneliden von vorn nach differenzirt. Der Nauplius ist eine typische Krebslarve; die Vorfahren der Krebse besassen noch keine typische Naupliuslarve, noch weniger stammen sie von einer naupliusähnlichen Stammform ab."

These conflicting views may now be compared with and tested by such evidence as can be obtained from the terrestrial groups.

As to the Insects, the conclusion reached was that they appear

to have been derived from an ancestor with more than ten segments, for reduction rather than increase of this number is observed. The reverse appears to be true of the Myriapoda, while Peripatus throws no light upon this point. In the Arachnida concentration also apparently occurs.

The general resemblance of the adult Peripatus to the Annelids, and the presence of segmentally arranged nephridia having the same function as in the Annelids, have given much support to the Annelid theory. Perhaps the most patent reason for tracing the Arthropoda to the Annelids, however, is the structure of the body. As the Arthropods are composed of a series of metameres each bearing a pair of appendages, this fact demands that the group should be derived from metameric forms. The only group which seems to fulfil all the requirements in this regard is the Annelida, and additional weight to this view is given by the presence in both groups of a neurocord (Giant Fiber) present in the nerve-cord.

To summarize the present status of scientific opinion. Many regard the Arthropoda as descended from an Annelid stock, and reject the Nauplius larva of Crustacea as being only secondary. Others reject the Annelid theory, at least so far as the Crustacea are concerned, regarding the Nauplius as a phylogenetic ancestral form, with some secondary modifications. They are also inclined to regard the Arthropoda as a polyphyletic group. A third view is that the Arthropods are derived from some unsegmented worm; while a fourth is that the Crustacea, Arachnida, Myriapoda, Peripatus and Insecta are independent groups of equal value.

Without continuing this line of thought farther here, we turn to the consideration of certain features which may aid in phylogenetic speculation. The tracheæ, not being present in any group simpler than the Arthropoda, as such, must be modifications of organs having some other function in the ancestors of the Tracheates, as it is inconceivable that rudiments of tracheæ should be of sufficient value for respiratory purposes to their possessors that they should be preserved. Regarding the Tracheates as derived from the Annelids, the opinion was at first held that these organs were modified nephridia, the function of which had been usurped by proctodæal diverticula—the Malpighian tubules.

This view has been negatived by the discovery that the nephridia are derived from the mesoblast, while the tracheæ are epiblastic, and by the coexistence of nephridia and tracheæ in Peripatus. Moseley has suggested that the tracheæ are homologous with the cutaneous glands of Bipalium, Hirudo, etc., while Sedgwick ('84) advances the hypothesis that "the tracheæ were at first simple pits of ectoderm in a diploblastic animal, and they gradually became more complicated and branched, as the other organs also became more complicated and folded."

If, as has been indicated above, the tracheæ of the Arachnids have arisen as modifications of the lung-books, they must either have originated independently from those of the other Tracheates, or these groups are derived from the Arachnids; the reverse —that the Arachnids are derived from the other Tracheates— being disproved by their crustacean affinities and in other ways. The derivation of the other Tracheates from the Arachnids cannot be accepted either. Then in Arachnids and Diplopods the stigmata open in the ventral plates; in the Hexapods and Chilopods, outside these plates, and in Peripatus they are scattered over the entire surface of the body. These differences of distribution may indicate an independent origin of the tracheæ in the different groups. Emery suggests (Grassi, '86 *c*, *Am. Nat. Review*) that if the tracheæ have arisen from diffusely distributed cutaneous glands, their openings might be supposed to unite with those of the nephridia and thus gain a segmental arrangement.

The origin of the Malpighian tubules has also attracted considerable attention. One view—that they are homologous with tracheæ—has already been stated. Another is that they are modified nephridia, but this view has recently been rather laid aside. Beddard ('89), however, has recently described an Annelid—Acanthodrilus—having irregularly located cæcal diverticula of the gut which become continuous with tubes lying in the nephridial network and not distinguishable from the nephridia. To change these to ordinary Malpighian tubules, Beddard states that it would only be necessary to limit their number and arrange them regularly. Their absence in Peripatus would be explained by the assumption that this secondary relation had not been established in that group.

The presence of similar tubes derived from the epiblast and from the hypoblast is a difficulty, however, and leads to a suspicion that these excretory tubes may have been independent developments, with no true homology existing between them.

The origin of the coxal glands has been treated of by Eisig ('87). From an examination of the skin and ectodermal glands of the Capitellidæ, he believes that from the spinning glands of Annelids have developed the Annelid bristle glands and the different Arthropod structures enumerated above as being considered homologous by him.

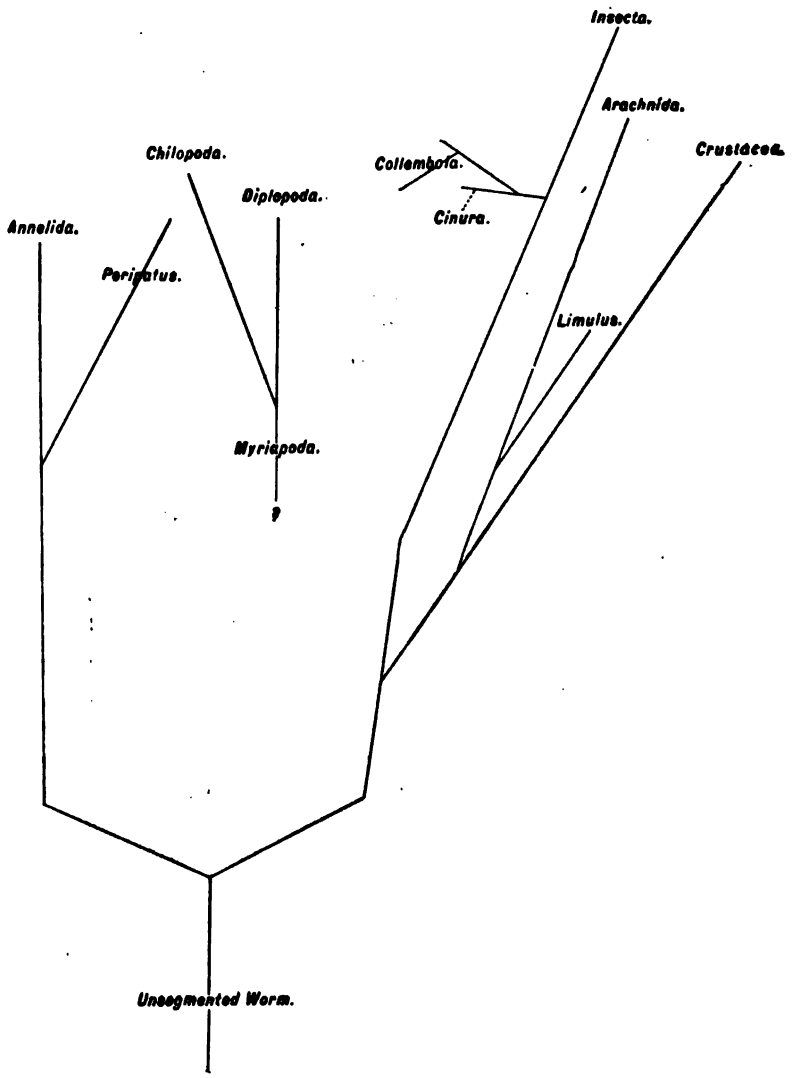
Following the article by Gulland ('85) on the coxal glands of Limulus is a note by Lankester on the homologies of these glands. He concludes that the coxal glands of Limulus have the essential features of nephridia, like those of Peripatus and the Chætopoda. He also regards the green glands of Crustacea as modified nephridia, and thinks that the sexual ducts of Arthropods should probably be also so considered.

Eisig ('87) only partially agrees with this view. As already stated, he derives the coxal glands from spinning glands, while he believes that from the Annelid nephridia have developed the salivary glands and sexual ducts in Annelids, Peripatus, Myriapods, Scolopendrella, Thysanura, Insects and Arachnoids, and probably also the weapon glands of the Myriapods and Insects.

One tendency of morphology at the present time seems to be to seek for universal homologies, oblivious of the principle termed by Darwin analogical variation. The argument of Hatschek given above is an illustration of this tendency. Darwin, in his Origin of Species, says, under this heading: "The members of the same class, although only distantly allied, have inherited so much in common in their constitution that they are apt to vary under similar exciting causes in a similar manner; and this would obviously aid in the acquirement through natural selection of parts or organs strikingly like each other, independently of their direct inheritance from a common progenitor."

The great differences of opinion as to the origin and relationships of the Arthropoda may, perhaps, become reconciled through this view, and homologies no longer be sought for between structures which are only analogous. If in some unsegmented worm

a slight variation should occur, the resulting branches might remain under nearly the same conditions and would therefore possess nearly the same structure. Hence we might naturally expect that development along parallel paths should follow. If subsequent changes of environment occurred, affecting these branches in the same manner and degree, the most favorable variations ensuing would tend to be preserved, and these would



probably be almost the same in the different branches. In this way might independently be developed the segmentation of the body, the general structure of the nerve chain, and other features common to Annelids and Arthropods. If now changes of environment should occur, differing between the groups, this would result in the appearance of different variations, of which those most nearly alike would, perhaps, no longer be most beneficial to all the groups, and in consequence different variations would be preserved by natural selection. These divergent features once present, farther changes of environment would be followed by a rapid divergence of the groups.

This view has little or no direct evidence in its favor, but in the search for homologies it should not be forgotten that such an analogy is at least conceivable, and if not so far-reaching as here hypothetically assumed, it may at least explain some of the less important similarities.

If this hypothesis were to be fully accepted, the relations of the groups concerned would, perhaps, be that expressed in the above diagram, in which ascending diverging lines represent diverging groups with increasing complexity, the horizontal lines little variation, and descending lines a degradation. The branches of the Crustacea, Insecta, Arachnida and Annelida have not been developed in the diagram.

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EXPLANATION OF PLATES.

Reference Letters.

ab. app. Abdominal appendage.
al. m. Alary muscles.
An. Anus.
ant. n. Antennal nerve.
ao. Aorta.
B. C. Buccal cavity.
b. m. Basement membrane, or tunica propria.
b. n. Buccal nerve.
bl. c. Blood corpuscles.
bl. s. Blood sinus.
Br. Brain.
cal. Calyx.
c. c. Crystalline cone.
c. m. Circular muscles.
co. Cornea.
cor. Corneagen.
c. r. Cell row.
c. t. Connective tissue.
cu. Cuticle.
cut. Cuticula.
d. b. Dim band.
d. ph. Dilator muscles of the pharynx.
e. d. Ejaculatory duct.
ep. Epithelium.
ex. External limiting membrane.
F. G. Fore gut.
g. c. Ganglion cell.
g. r. Germinal ridge.
H. Heart.
H. G. Hind gut.
h. s. Härchensaum.
hy. Hypodermis.
K. m. Krause's membrane.
l. b. Light band.
l. m. Longitudinal muscles.
m. Muscle fibers.
m. a. Muscle attachment.
md. Mandible.

m. ep. Molted epithelium.
mes. Mesentery.
M. t. Malpighian tubule.
M. G. Mid gut.
mx. Maxilla.
n. Nerve.
n. c. Nerve cell.
nuc. Nucleus.
oc. Ocellus.
oc. n. Ocellar nerve.
œs. Œsophagus.
op. Ovipositor.
op. l. Optic lobe.
op. n. Optic nerve.
Os. Ostium.
ov. Ovary.
ova. Ova.
ovid. Oviduct.
p. Pigment.
pal. Palpus.
p. a. o. Post-antennal organ.
p. c. Pigment cells.
Ph. Pharynx.
Ret. Retinula.
Rh. Rhabdomere.
r. s. Receptaculum seminis.
s. o. Sense organ.
s. œ. g. Sub-œsophageal ganglion.
sp. Spermatozoa.
t. b. Tactile bristle.
t. p. Tunica propria or basement membrane.
V. Valve.
vac. Vacuoles.
vag. Vagina.
v. d. Vas deferens.
Vit. Vitrella.
v. n. Ventral nerve cord.
y. Yolk.

The figures were drawn with a Zeiss-Oberhäuser camera and a No. 4 eyepiece. The Zeiss objective used is stated in each case below. The drawings have been reduced in the process of printing nearly one-half, but were drawn with a view to a reduction of but one-third. Figs. 1 to 63, inclusive, are of Anurida maritima; the remainder are of Lepisma saccharina.

PLATE XLVIII.

FIGURE 1.—Surface view of the external cuticula. D.

FIG. 2.—Section of hypodermis and cuticula. D.

FIG. 3.—Vertical, slightly oblique section of the front of the head. The jaws are cut just behind their tips, and the pharynx is cut almost tangentially. Slightly diagramatic. C.

FIG. 4.—Longitudinal section of the œsophagus. D.

FIG. 5.—Cross section of the œsophagus. D.

FIG. 6.—Attachment of two muscles to the body wall, showing th modification of the hypodermis at that point. D.

FIG. 7.—Fat body in cross section of the animal, on one side. D.

FIG. 8.—Muscle of a leg, showing light and dim bands and Krause's membrane. D.

FIG. 9.—Maxilla. D.

FIG. 10.—Mandible. D.

FIG. 11.—Surface view of the mid-gut, showing the relations of the muscle fibers and the epithelium. D.

FIG. 12.—Longitudinal section of the valve between the œsophagus and mid-gut. D.

FIG. 13.—Cross section of the same. Larger specimen. D.

FIG. 14.—Cross section of the mid-gut in the mesothorax, showing the molted epithelium not yet thrown off. D.

FIG. 15.—Cross section of the mid-gut in the third abdominal segment. D.

FIG. 16.—Longitudinal section of the hind-gut and the valve between it and the mid-gut. D.

FIG. 17.—Cross section of the hind-gut. Slightly diagramatic. D.

FIG. 18.—Longitudinal section of the posterior end of the heart, showing its connective tissue-attaching thread. C.

FIG. 19.—Cross section of the heart close to a pair of ostia which show faintly through the walls. $\frac{1}{12}$ in. immersion.

FIGS. 20-27. Series of camera outlines showing the relations of heart and aorta to the gut. 20, in the metathorax; 21, in the mesothorax; 22, in the prothorax, at the valve between the fore and mid-guts—the heart has now become the aorta; 23, just in front of the prothoracic legs; 24, at the front of the prothorax; 25, at the back part of the head; 26, a little farther forward; 27, just behind the brain. C.

FIG. 28.—Blood corpuscles. D.

FIG. 29.—Gland cells in the dorsal posterior portions of the head. D.

FIG. 30.—Duct leading from these cells to the lower lip. D.

FIG. 31.—Opening of this duct upon the ventral surface of the lower lip, at the posterior end of the median cleft. Not quite longitudinal, hence not showing the cleft. $\frac{1}{12}$ in. immersion.

FIG. 32.—Cross section of the median ventral tube leading from the opening shown in Fig. 31 to the abdominal vesicle, in the region of the head. D.

FIG. 33.—Cross section of the same, in the mesothorax. D.

FIG. 34.—Cross section of the abdominal vesicle. The cells have separated from the chitin cuticula which covers them. D.

PLATE XLIX.

FIG. 35.—Cross section of the brain, cutting the optic lobes and optic nerves leading to the ocelli, and showing the relation of the brain to the œsophagus. D.

FIG. 36.—Cross section of the brain, in front of the optic lobes. D.

FIG. 37.—Vertical longitudinal section of the brain, on one side, cutting the root of the antennal nerve. D.

FIG. 38.—Longitudinal section of a tarsal ganglion. D.

FIG. 39.—Cross section of the metathoracic ganglion, showing the nerves to the metathoracic feet. D.

FIG. 40.—Surface view of the metathoracic ganglion, from above, with its main nerve trunks. D.

FIG. 41.—Surface view of part of the left side of the upper surface of the head, showing the post-antennal organ and the ocelli in their relative positions. D.

FIG. 42.—Vertical section of an ocellus. $\frac{1}{12}$ in. immersion.

FIG. 43.—Vertical section of a post-antennal organ. The pigmented tissue of the organ has been removed. F.

FIG. 44.—Surface view of a post-antennal organ. D.

FIG. 45.—Section of the ventral portion of the fourth abdominal segment, showing the posterior nerve cord and the rudiments of the spring. D.

FIG. 46.—Left antenna, showing sense bristles and sense organ. C.

FIG. 47.—Section of the tip of an antenna. The hypodermis has shrunk away from the cuticula, but without breaking the nerves running to a sensory bristle and to the sense organ. D.

FIG. 48.—Camera outline, showing the form of the sense organs. The dotted line is seen on focusing deeper. D.

FIG. 49.—Cross section of the testis of an immature male. D.

FIG. 50.—Cross section of a nearly mature testis, cut about on the line AB, Fig. 58. D.

FIG. 51.—Longitudinal section of the vas deferens and of the genital papilla of an immature male. D.

FIG. 52.—Cross section of the vas deferens. D.

FIG. 53.—Longitudinal section (somewhat tangential) of the vagina, oviduct, receptaculum seminis and a portion of the ovary. D.

FIG. 54.—Cross section of the oviduct and receptaculum seminis. D.

FIG. 55.—Cross section of the ovarian tube, showing strings of germinal cells. Young female. D.

FIG. 56.—Longitudinal section of the anterior end of the ovarian tube, showing germinal epithelium. D.

FIG. 57.—Longitudinal section of part of an adult ovarian tube, showing developing ova. D.

PLATE L.

FIG. 58.—Longitudinal section of the testis, showing the changes in maturation of spermatozoa from the condition shown in Fig. 50, to the adult, showing seminal globules. D.

FIGS. 59–62.—Cross sections of a developing testis, 59 representing a stage intermediate between 49 and 50; 60, a cut about along the line CD, Fig. 58; 61, a cut about along the line EF, and 62, a cut about along the line GH.

FIG. 63.—Longitudinal section of the vas deferens of an adult male. D.

FIG. 64.—Ovipositor and posterior end of abdomen of Lepisma. After Grassi.

FIG. 65.—Scale of Lepisma. D.

FIG. 66.—Section of hypodermis and cuticula, showing attachments of scales. D.

FIG. 67.—Section of cuticula and hypodermis at the juncture of the prothorax and mesothorax on the dorsal surface, with a muscle attached. D.

FIG. 68.—Cross section of a muscle bundle. A.

FIG. 69.—Mandible. A.

FIG. 70.—Maxilla, with the basal joint of the maxillary palpus.

FIG. 71.—Labium. After Grassi.

FIG. 72.—Cross section of the pharynx. C.

FIG. 73.—Cross section of the wall of the œsophagus. D.

FIG. 74.—Cross section of the wall of the stomach, with a Malpighian tube in section, just external to it. D.

FIG. 75.—Cross section of the ileum, with three of its supporting mesenteries. C.

FIG. 76.—Section of one of the folds of the rectum, showing the histological structure of its walls. D.

FIG. 77.—Longitudinal section of a Malpighian tube, showing yellowish granules. D.

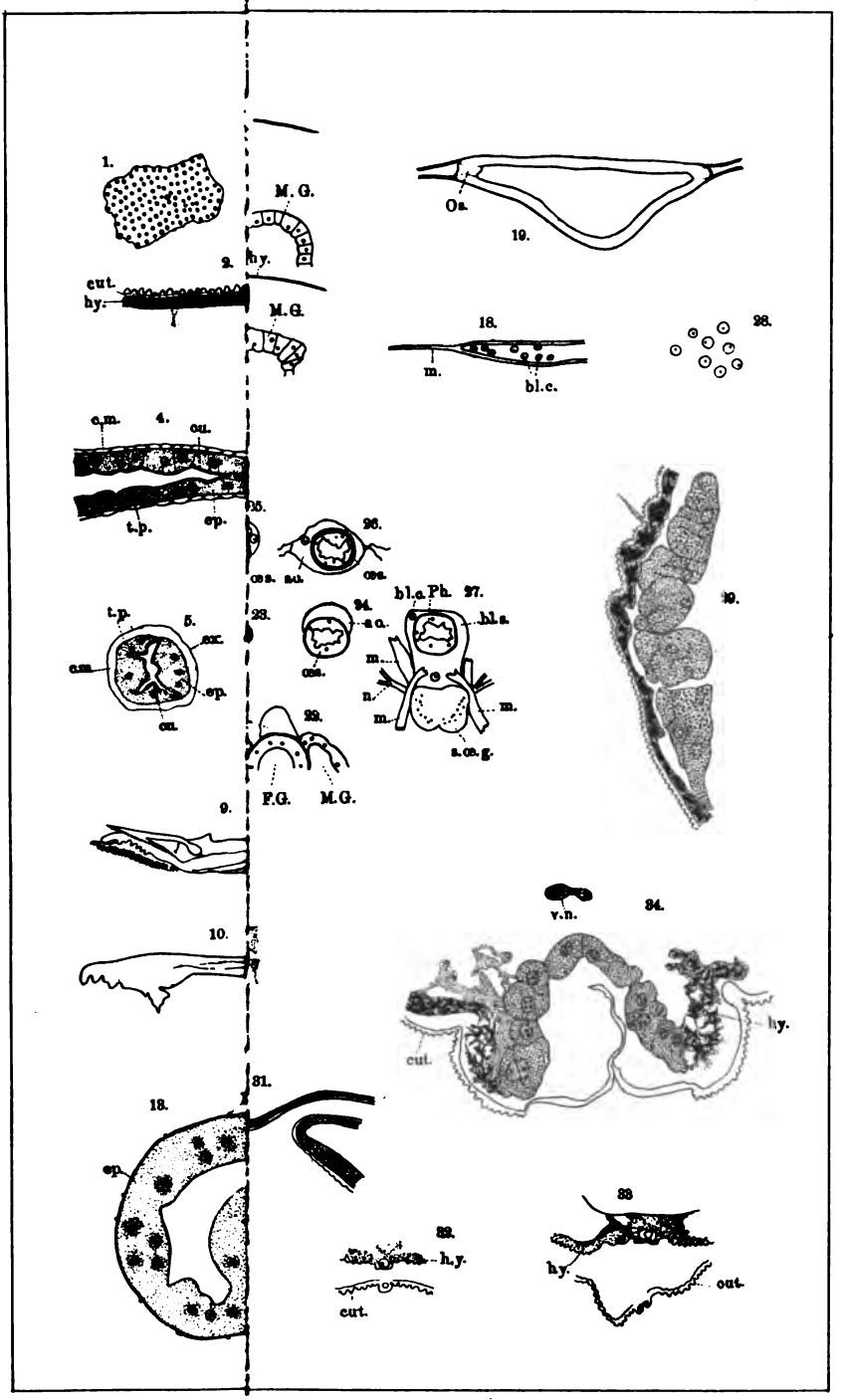
FIG. 78.—Section of a dense portion of the fat body, showing also the hypodermis and a tactile bristle. D.

FIG. 79.—Cross section of the heart in the posterior region of the abdomen. D.

FIG. 80.—Cross section of the heart in the metathorax, showing blood corpuscles and nuclei of the muscles of the walls. D.

FIG. 81.—Longitudinal section of the metathoracic and first two abdominal nerve ganglia. A.

FIG. 82.—Longitudinal horizontal section of the posterior end of



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the nerve cord, showing the sixth, seventh and eighth ganglia. After Grassi.

FIG. 83.—Cross section of the protocerebron, showing the antennal nerve and cutting the anterior edge of the optic ganglion. C.

FIG. 84.—Vertical section through two ommatidea of the compound eye. One ommatideum has been depigmented; the other has the pigment left in place.

FIG. 85.—Diagram of the parts of an ommatideum separated from its main axis and projected onto a plane, the more superficial layers being external to the deeper layers. Two of the cells of the retinulæ have pigment: it is not represented in the other cells.

FIG. 86.—Diagram of the anatomy of the adult male genital organs. After Grassi.
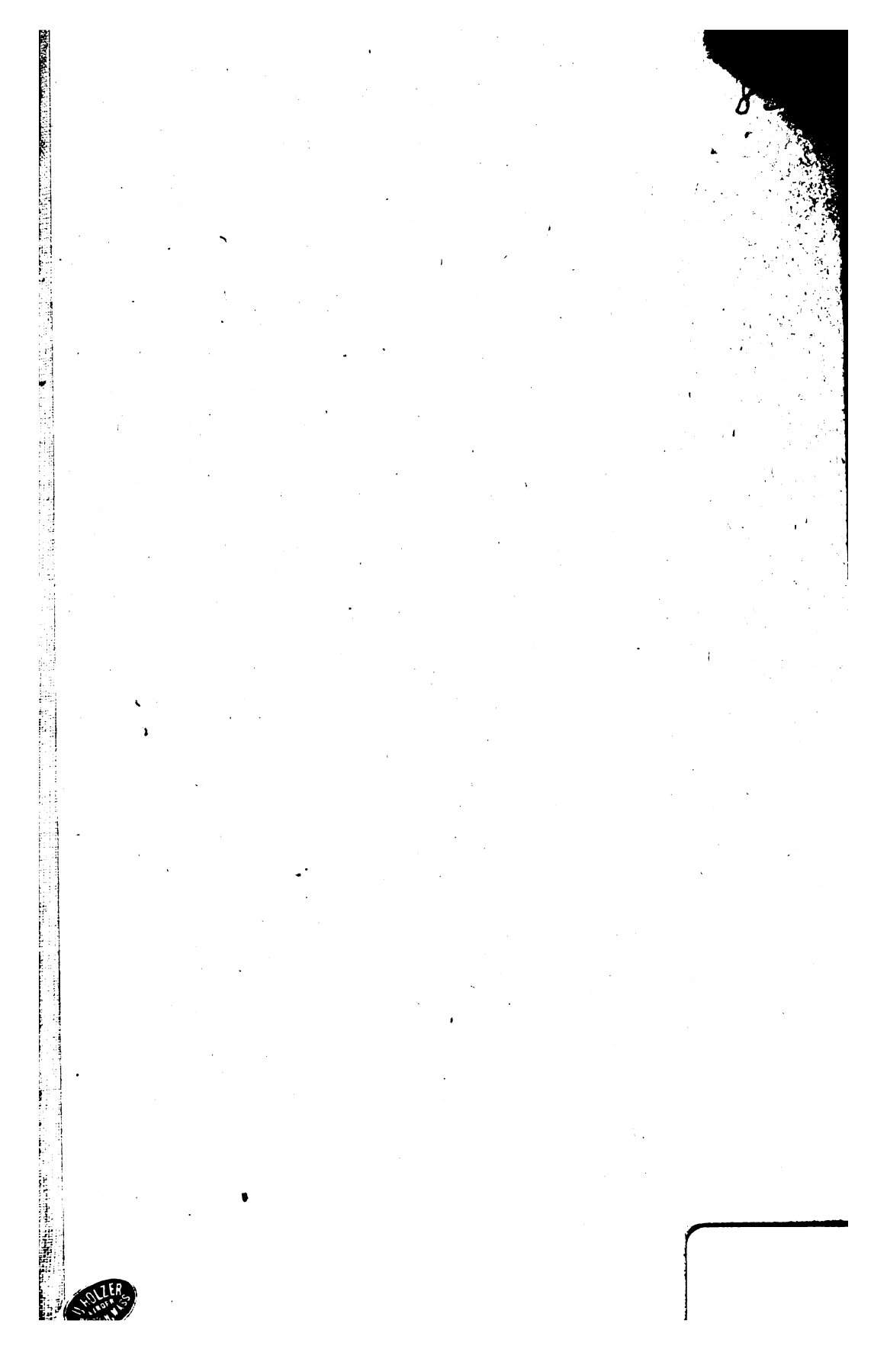
FIG. 87.—Diagram of the anatomy of the genital organs of an immature female. After Grassi.